THE RESPONSES OF THE STOMATA OF CONIFERS TO HUMIDITY AND WATER POTENTIAL

by

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B.Sc. (Lanc.)

A thesis submitted in fulfilment of the requirements

for the degree of Doctor of Philosophy

to the

University of Edinburgh

1984

ABSTRACT

The responses of stomatal conductance (g_s) and CO_2 exchange (A) of a range of conifers to leaf-to-air vapour pressure difference (D) were investigated, using gas exchange techniques. Scots pine, Sitka spruce, lodgepole pine and hybrid larch were studied. The possibilities that there might be interactions between these responses and those to photon flux density, temperature and water potential were also investigated.

Responses of g_s to D for the different species ranged from no response for 10-month-old Scots pine shoots, to a decrease in g_s, for 3-month-old Sitka spruce shoots, to a degree that caused E* to reach an asymptote as D was increased. However, almost as much variation was found between shoots of different ages, of the same species. In no experiment was E found to decrease as D was increased and thus there was no requirement to invoke a 'direct' response of the stomata to D. A was found to decline linearly as D was increased, the decline being stronger for plants with stronger stomatal responses to D.

For Scots pine shoots, with virtually no response of g_s or A to D at high photon flux density, the sensitivity of g_s and A to D did not increase at lower photon flux densities.

The response of g_s to D, for Sitka spruce, was virtually independent of temperature, although g_s did increase slightly in response to higher temperatures. A also increased in response to temperature, but declined linearly with increasing D at all temperatures. Intercellular space, CO_2 mole fraction (C_i) appeared to be independent of temperature, but declined as a result of the decrease in g_s, as D was increased.

For both Scots pine and Sitka spruce the response to water potential and D was studied by withholding water. The response of g_s could be adequately described by a model which assumes no interaction between the two variables. By studying A/C_i curves, it was shown that there were no direct effects on the photosynthetic mechanism of moderate declines in water potential. Thus stomatal limitation of A increased as g_s decreased in response to declining water potential.

* E = the transpiration rate

For all the experiments, there was evidence for a decline in A, as D was increased, that could not be attributed to stomatal closure. This was shown as A declined, when D was increased, even though C_i remained virtually constant. The cause of this could not be explained.

For several of the experiments dE/dA was calculated using the models that had been derived to fit the data. These values of dE/dA were used to test the hypothesis of Cowan (1977, Adv. Bot. Res 4: 117-228) that the stomata should respond in an "optimal way" to changes in D, by maintaining dE/dA constant. In the cases of lodgepole pine and hybrid larch, dE/dA was more or less constant, but in the majority of the other experiments it was not.

ACKNOWLEDGEMENTS

I would like to thank Professor P. G. Jarvis, my supervisor, for his stimulating advice and guidance throughout this project.

I would like to thank Dr. J. I. L. Morison for his introduction into the art of gas analysis, during the early stages of this work.

I would also like to thank my wife, Jane, for her patience and support. In particular I would like to thank her for the help she gave me in the preparation of this thesis.

This work was carried out with the aid of a research studentship from the Natural Environment Research Council.

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INTRODUCTION

1.1 General introduction

"Although stomatal behaviour is very sensitive to the turgor relations of the plant, it is comparatively unaffected by changes in the relative humidity of the ambient air."

The above quotation comes from the well-respected textbook written by Meidner and Mansfield in 1968. The quotation reflects a "blind-spot" in the ideas of stomatal physiologists which developed in the late 1950's and was maintained until the early 1970's. It is not clear why the possibilty of a response to humidity was overlooked: perhaps it was overshadowed by intensive research on the response of stomata to water stress. However, many early plant physiologists had proposed such a response e.g. Haberlandt (1914, 1884 1st edition) stated "The majority of stomata are exceedingly sensitive to any fluctuations in the humidity of the atmosphere". Another reason for the confusion was, undoubtedly, caused by the variation in response now known to occur between species and even within species, an example of which is the variation in response found for *Zea mays* L. (Raschke & Kuhl, 1969; Raschke, 1970).

In the 1970's, the studies of Lange *et al* (1971) and Lösch (e.g. Lösch, 1977), however, confirmed, by experiments on isolated epidermis, that the stomata are capable of responding directly to changes in the ambient humidity. In retrospect, many earlier experiments may also have shown a response to humidity (see Chapter 3 for a detailed discussion of the literature with reference to conifers). Over 70 species have now been found to show some degree of stomatal closure as the leaf-to-air vapour pressure difference (D) increases (Sheriff, 1977; Lösch, 1979a). However many other species have been shown not to respond to D, for example as reported by Rawson *et al* (1977). A small number of species have even been shown to open their stomata as D increases (Sheriff, 1977).

Some of the responses of g_s to D reported are so strong that transpiration from the leaf (E) when plotted as a function of D reaches a maximum and then declines as D increases further, e.g. Schulze *et al*

(1972). This type of response cannot be simply explained by a mechanism which involves feedback from E onto the stomata, via changes in leaf water potential. Using a theoretical approach, Farquhar (1978) showed that such a response must involve direct sensing of D outside the leaf, possibly by evaporation from a site in the epidermis. Taking a term from engineering control theory he called this a "feedforward" or direct response to D.

This type of response is of particular interest to stomatal physiologists, as it implies that the response to D is not simply an extension of the well-studied water relations of a plant, but must involve a separate mechanism by which the stomata are controlled. A direct response of g_s to D is also likely to be of significance to the water balance of plants in the field, and may therefore vary in its strength with the evolutionary adaptation of different species to different environments.

1.2 The objectives of this thesis

One of the best demonstrations of a direct response of g_s to D was shown by Ng (1978) for *Pinus sylvestris* L.. See also Jarvis (1980) and Jarvis and Morison (1980). The initial objectives of this thesis were:

i) To see if such a response occurred in any other conifers, particularly some of those important in British commercial forestry.

ii) To estimate the consequent reduction in assimilation (A) caused by stomatal closure in response to D.

iii) To try to gain greater understanding of the mechanism involved in the response, by studying the interactions between the response to D and to light, temperature and in particular water potential.

iv) To test the hypothesis proposed by Cowan (1977) that the stomatal response to D was a significant component of his hypothesis of optimal stomatal action and was instrumental in

maintaining dE/dA constant.

1.3 General approach

These objectives have been approached by studying shoots of potted seedlings in a controlled environment, assimilation chamber. Thus the responses of g_s and A could be studied to one variable alone, whilst all other variables were controlled. This approach was preferred to making measurements in the field as it is much easier to define the individual physiological responses that are of interest. One does, however, have problems in extrapolating such laboratory experiments to describe how the plant will respond in the field.

Experiments are described where the responses of g_s and A to D have:

i) been measured for a range of species (Chapter 3),

ii) been tested to see if there is any interaction with photon flux density (Chapter 5),

iv) been tested to see if there is any interaction with temperature (Chapter 7),

iv) been tested to see if there is any interaction with bulk leaf water potential (Chapters 8 & 9).

The results of these experiments were used to test the hypothesis that stomata respond to D to maintain dE/dA constant (Chapter 10). Then, in the light of the results presented in previous chapters, possible mechanisms for the response of stomata to D are discussed in Chapter 11.

CHAPTER 2

MATERIALS AND METHODS

2.1 Introduction

Much of the equipment and experimental techniques used have previously been described by Ng (1978) and Morison (1980). To avoid unnecessary repetition the reader is referred to those theses. Details are given below of methods where they either differ from those previously used, or where the details are critical to the results obtained or their analysis.

2.2 Plant material

The plants used were either potted seedlings or cut shoots taken from trees around the university campus. The age of the material and pretreatment varied from experiment to experiment, therefore specific details are given in the relevant chapters.

All potted seedlings were grown, unless otherwise stated, in a peat/sand mixture (University of California 2Cd mixture, Matkin & Chandler, 1957) in plastic pots. The plants were grown outside and were brought into either the glasshouse or growth rooms, at least six weeks prior to the experiments. During the winter months plants were brought in to the glasshouse to induce early bud break and provide new shoots to allow work to continue.

Three types of pretreatment conditions were used, in all cases with a daylength of 16 hours:

i) An unheated greenhouse in which the day length could be extended using mercury vapour lamps (Thorn, 400 W MBIF), providing a photon flux density of approximately 300 μmol m^{-2} s^{-1}, at the level of the shoots which were studied. Saturation vapour pressure deficit and air temperature were mainly uncontrolled, although

ventilation was increased if air temperature exceeded 20 °C.

ii) A walk-in growth room with illumination provided by a combination of mercury vapour (Thorn Kolarac 400 W) and tungsten light bulbs. The photon flux density was approximately 300 µmol m^{-2} s^{-1} at the level of the shoots which were studied. Day and night air temperatures were 20 °C. Relative humidity was controlled at 75% which is approximately equivalent to a saturation vapour pressure deficit of 0.6 kPa.

iii) A Fison's 2300 growth cabinet with illumination provided by a combination of mercury vapour (Wotan 250 W, HQI-NDL) and tungsten light bulbs. The photon flux density was approximately 750 µmol m^{-2} s^{-1} at the level of the shoots which were studied. Day and night temperatures were 20 °C. Relative humidity was controlled at 75%, equivalent to a saturation vapour pressure deficit of 0.6 kPa. However, because of the high radiation, leaf temperatures were estimated to be approximately 3 °C above air temperatures so that the leaf-to-air vapour pressure difference was approximately 1.1 kPa during the light period.

To increase legibility all references to species of conifers in this thesis are made using the 'common' English name. A full list of the 'common' names with their scientific latin names and sources are given in Appendix 2.

2.3 Gas exchange measurement

For measurement of the rates of influx/efflux of CO_2, and transpiration of a shoot, an 'open' gas exchange system was used (Jarvis, 1971). The system was originally developed by Ludlow and Jarvis (1971), and has been described by Leverenz (1978), Ng (1978) and Morison (1980). Several modifications have been made since, so a brief description is necessary. A block diagram of the analysis system is shown in fig. 2.1

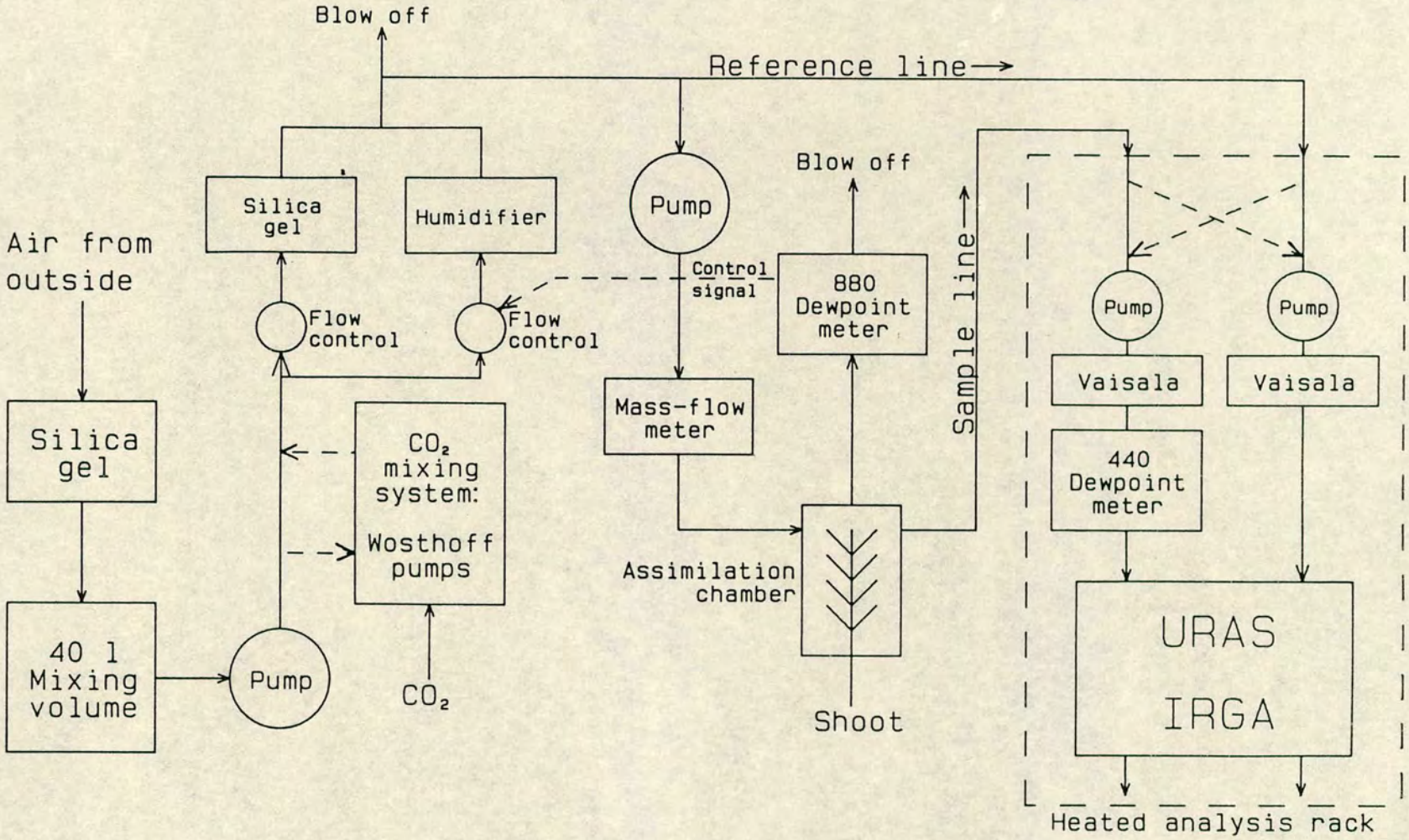

Figure 2.1: A block diagram of the gas analysis system. The arrows indicate the direction of gas flow. Dashed lines indicate optional pathways.

2.3.1 Air conditioning

Air was taken from outside the building at a height of *ca* 25 m and passed through a column of silica gel, prior to entering a mixing vessel with a volume of 0.04 m^3. Thus the air was moderately dry prior to being conditioned. The air was then taken through CO_2 and/or water vapour conditioning systems. In the CO_2 system the air was scrubbed free of CO_2 and mixed with pure CO_2, using a series of three Wosthoff gas-mixing pumps, to produce CO_2 concentrations from 0 - 1000 $\mu mol\ mol^{-1}$. However, unless otherwise stated in the relevant chapters, ambient outside air was used in the majority of experiments, i.e. with a CO_2 mole fraction of approximately 340 $\mu mol\ mol^{-1}$. Although ambient CO_2 was found to vary by up to $\pm 20\ \mu mol\ mol^{-1}$, for most experiments it was thought that this variation would not affect the responses of the stomata, as the stomata of conifers have previously been shown to be relatively insensitive to changes in C_a in this range (Morison, 1980).

In the water vapour conditioning system, the air stream was split and either further dried with silica-gel or humidified by passing through jars containing moist filter paper. The humidification jars were positioned in a cabinet at a temperature of 35 °C. The flow rates of air through the two systems, and thus the resultant vapour pressure, was controlled manually by two flow controllers (GEC-Elliot Model 1100 Rotameters).

In September 1981 the flow controller for the humidification system was replaced by a Tylan electronic, mass-flow controller (range 2 - 85 $cm^3\ s^{-1}$). This allowed either finer manual control or completely automatic control of the water vapour pressure in the cuvette (see below).

The two gas streams were then mixed and passed through a mixing jar of 1000 cm^3 volume. The gas line was then split. A precisely known flow rate of air passed through a Brook's mass-flow controller (Model No. 5810) to the cuvette containing the shoot being studied. Air was also taken directly to the rack containing the gas analysis instruments where it was used as a reference.

Air passing into the cuvette was mixed by a fan. Most of the air leaving the cuvette passed out of a 'blow off'. In May 1981 a Model 880, Cambridge Instruments dewpoint meter was installed in the line leading to the 'blow off'. This allowed continuous monitoring of the vapour pressure of the air leaving the cuvette, independent of any calibration procedures taking place in the measurement rack.

In September 1981 an Eurotherm model 071, two-term controller was installed. This used a conditioned signal from the dewpoint meter to control the mixing of humid and dry air in the humidity conditioning system, via the Tylan mass-flow controller. Thus the vapour pressure in the cuvette could be held at a fixed, predefined level, independent of changes in the rate of transpiration of the leaf that might otherwise act to alter the vapour pressure within the cuvette, because of its small volume.

2.3.2 **Assimilation Chamber Design**

The brass cuvette was modified by replacing the original "Perspex" windows with 6 mm plate glass, to reduce problems of absorption and adsorption of water vapour and CO_2 by components of the chamber (Dixon & Grace, 1982; Bloom *et al*, 1979). Leaf temperature was held at a constant level of 20 $\pm$0.1 ^{o}C (unless otherwise stated) by cooling the chamber with a Peltier device. Under conditions of high radiation, low stomatal conductance and high humidity there was a risk of condensation forming on the cold surfaces of the chamber. To minimise this effect, the heat load on the chamber was reduced by adding further insulation, in the form of extra layers of expanded polystyrene, on the external metal surfaces of the chamber. The windows of the chamber were also double glazed by adding an outer layer of 6 mm "Perspex", separated from the glass by an air gap of 5 mm.

2.3.3 **Illumination**

The cuvette was illuminated bilaterally as described previously by Leverenz & Jarvis (1979) with 400 W, Wotan HQI-T light sources (see Morison (1980) for spectral details). These bulbs subjected each side of the chamber to photon flux density of *ca* 500 μmol m^{-2} s^{-1} (a total of at least 1000 μmol m^{-2} s^{-1}). This level of illumination was used for all experiments, unless otherwise stated, as it was intended to work mainly at light saturation (for stomatal opening; see Ng, 1978).

2.3.4 **Gas analysis**

The reference and sample gas lines were taken into a heated rack where they entered a solenoid-valve switching system which allowed the gas pathways to be redirected for calibration etc. All tubing in this system was replaced with Samuel Moore & Co., Dekabon 1300, laminated tubing to reduce the time taken for calibration, as the older PVC tubing took a long time to equilibrate with the gas flowing. The laminated tubing was impermeable to CO_2 and had very low water absorption properties. In addition the sampling pumps were reconfigured so that the gas pressure in the lines did not exceed atmospheric pressure and thus risk condensation at high water vapour pressures.

2.3.5 **Water vapour measurement**

The dual Vaisala sensor system, as described by Morison (1980), was used both for monitoring and measurement. All data presented, unless stated otherwise, are from measurements with these sensors. Prior to starting any experiments the sensor circuits were modified to improve stability with changing temperature, and the thermocouples measuring the sensor temperatures were fixed on the non-sensing surface of the sensor for more accurate measurement.

Results from the Vaisalas were always checked against readings from a Model 440 Cambridge Instruments dewpoint meter placed downstream in the

gas line (see fig. 2.1). However, only one dewpoint meter was generally available and as the water vapour pressure in the reference line was often changed to allow control the vapour pressure around the shoot, comparisons between the Vaisalas and dewpoint meter were only valid directly after a calibration sequence, during which the dewpoint meter measured the reference line vapour pressure.

2.3.6 **Carbon dioxide measurement**

The system for measuring carbon dioxide was unchanged from that described by Morison (1980). The zero and sensitivity of the analyser were checked between every measurement using the "tube-length method" of calibration (Parkinson & Legg, 1971).

2.3.7 **Data logging**

The data logging facilities, as described by Morison (1980), were used in all experiments to capture data onto paper tape which was then fed into the university's main-frame computer where data were processed using a Fortran program. This allowed one to take several readings at each treatment in an experiment, and mean values of at least 10 readings were calculated thus reducing error due to signal noise.

2.3.8 **Additional measurements**

Plan leaf area was measured using a Li-Cor LI-3100 planimeter as described by Morison (1980).

Xylem water potential was measured on needle fascicles with a small needle bomb (Roberts & Fourt, 1977) in the case of Scots and lodgepole pine (species which have long needles) and on cut shoots in a larger pressure chamber for species with short needles e.g. Sitka spruce. When using the needle bomb great care was taken to ensure that as large a proportion of the needle being measured as possible was enclosed in the

pressure chamber. If this is not done too low water potentials are measured (Ritchie & Hinckley, 1975).

For experiments in which the shoot was to be used again needles or side shoots were taken from the same branch as that being measured to sample water potential. These needles or shoots were enclosed in a black plastic bag, and were thus not subjected to the same evaporative demand. Their xylem water potential was not significantly different to that of the shoot in the measurement chamber, even after the measurement shoot had been subjected to a large vapour pressure deficit. (See Chapter 3 for further details.)

2.4 Calibration techniques

2.4.1 Flow meters

The flow of air entering the assimilation chamber has to be known accurately. Therefore, the mass-flow meter was calibrated against a range of bubble flowmeters (Levy, 1964). The volumetric flow calibration was converted to a molar flow of air, using the temperature and pressure of the gas in the bubble flowmeters at that time. As the output signal of mass-flow meters is actually directly proportional to the product of the molar heat capacity of the gas and the molar flow of gas, this technique removes the complication of correcting the conventional volumetric calibration of mass-flowmeters for changes in pressure and temperature on a day to day basis. No correction was applied to account for changes in the molar heat capacity of air at different moisture contents, as recommended by Leuning (1983), but the resultant errors in molar flow are less than ±0.15%, over the range of water vapour pressures used.

2.4.2 Water vapour

The water bath technique, as used previously, was used to calibrate the Vaisala sensors and dewpoint meter *in situ*. In addition, prior to

calibration, British Oxygen Company, oxygen-free-nitrogen (with a dewpoint < -50 °C) was passed through the analysis system, for at least 6 hours. This allowed a check for leaks in the system and provided a check of the zero reading of the Vaisala sensors and the cooling capacity of the dewpoint meter.

2.4.3 **Carbon dioxide**

As mentioned above the infra-red gas analyser was calibrated, prior to each set of measurements, using the "tube length" method (Parkinson & Legg, 1971). This technique, however, relies on the knowledge of the effective ratio of the short to long cell length and as this may vary with contamination of the cells or ageing of the windows, this ratio was measured, as follows.

Carbon dioxide-free air was mixed with pure CO_2, using a series of Wosthoff gas mixing pumps to provide a reference mole fraction in the range of normal ambient concentrations. A pump was then used to take a proportion of this and feed it into a WD600 A.D.C. gas diluter (Parkinson & Day, 1979). The diluter had previously been recalibrated using the bubble flowmeter technique to a relative accuracy of better than ±0.5% for each orifice. The diluter was then used to generate a range of CO_2 mole fractions less than the reference, over the range of differentials normally experienced during an experiment. Thus pairs of known CO_2 concentrations were then used to calculate the effective short to long cell length ratio by comparing the sensitivity of the analyser calculated from these gases with that calculated by flushing the short cell with CO_2 free air.

This technique was preferred as there is flowing gas in both reference and sample cells simultaneously. The accuracy is equal to that of using a series of cascaded Wosthoff pumps for which the absolute error of generating any one CO_2 concentration is typically ±2 μmol mol^{-1} (Morison, 1980). This can lead to substantial errors when trying to calibrate a differential of only *ca* 20 - 30 μmol mol^{-1}.

2.4.4 **Temperature**

Considerable problems were encountered in both temperature measurement and control, mainly as a result of fluctuations in room temperature. Part of the problem was traced to spurious thermal voltages in the thermocouple system. Particular grades of thermocouple wire were also found to deviate markedly from the specification. These errors resulted in the actual temperatures being as much as 1 °C above the value obtained from industrial thermocouple tables at 20 °C. Thus each thermocouple used, was calibrated, *in situ*, against a high accuracy platinum resistance thermometer (with a long-term absolute accuracy of ±0.02 °C), over the range of temperatures that were to be encountered. A linear regression was calculated giving an accuracy of better than 0.1 °C, in that range.

2.5 **Theory**

Initially the theoretical approach, as outlined by Morison (1980), was used to calculate stomatal conductance, transpiration, assimilation and internal CO_2 concentrations from the gas exchange data. After a few preliminary experiments (from which no data are presented in this thesis), the theory used was modified to take into consideration the correction factors proposed by Parkinson & Penman (1970) and fully derived by Jarman (1974). Approximately nine months after the start of experimentation the theory was modified to deal with molar fluxes, resulting in an approach similar to that outlined by von Caemmerer & Farquhar (1981).

2.5.1 **Water vapour fluxes**

For a shoot in an open gas exchange system:

$$E = \frac{F_{e_o o} - F_{e_e e}}{P\ L} \qquad 2.1$$

The symbols used are listed in Appendix 1.

Converting the water vapour partial pressures to mole fractions gives:

$$E = \frac{F_o w_o - F_e w_e}{L} \qquad 2.2$$

Where $$F_o = F_e + E L \qquad 2.3$$

Thus $$E = \frac{F_e (w_o - w_e)}{L (1 - w_o)} \qquad 2.4$$

Total conductance to water vapour can be calculated, including the correction for molecular interactions between water vapour and air as derived by Jarman (1974), from

$$E = g_t (w_i - w_a) + w_b E \qquad 2.5$$

Where $$w_b = (w_i + w_a) / 2 \qquad 2.6$$

Assuming that $w_o = w_a$, total conductance can be calculated as:

$$g_t = \frac{E (1 - w_b)}{(w_i - w_o)} \qquad 2.7$$

For sake of legibility and comparability with previous workers, the concept of a leaf-to-air vapour pressure difference (D) is retained in the text and graphs of this thesis.

$$D = e_i - e_a = P (w_i - w_a) \qquad 2.8$$

e_i (and w_i) were calculated assuming that the air was saturated at leaf temperature. No corrections were applied to account for low leaf water

potentials, but this would at worst have resulted in an error of +2% in e_i (w_i), for the range of water potentials covered (Milthorpe, 1962).

Assuming that the cuticular conductance to water vapour is very small and can be ignored,

$$\frac{1}{g_t} = \frac{1}{g_s} + \frac{1}{g_a} \qquad 2.9$$

and therefore

$$g_s = \frac{g_a - g_t}{g_t g_a} \qquad 2.10$$

Values of g_a were taken from the work of the previous workers who had used the same gas exchange system. For Scots pine and lodgepole pine a value of 12 mol m^{-2} s^{-1} was taken following Ng (1978). For the smaller, but more densely foliated shoots of Sitka spruce, hybrid larch and Douglas-fir a lower value of 10 mol m^{-2} s^{-1} was taken following Morison (1980).

2.5.2 CO_2 fluxes

In a similar way as for E above, it can be shown that:

$$A = \frac{(C_e - C_o) F_e (1 - w_o)}{L (1 - w_e)} \qquad 2.11$$

Unlike the procedure followed by von Caemmerer & Farquhar (1981) the air was not dried prior to passing into the infra-red gas analyser thus further corrections did not have to be made to equation 2.11. Previous workers have shown that the sensitivity to water vapour of the analyser used in this laboratory was insignificant.

2.5.3 Internal CO_2 concentration

Following the same arguments, a relationship analogous to equation 2.5 can be derived for A, i.e.

$$A = g_{tc}(C_a - C_i) - C_b E \qquad 2.12$$

Where

$$C_b = (C_a + C_i) / 2 \qquad 2.13$$

The total conductance to CO_2 (g_{tc}) can be calculated from g_s and g_a by applying the ratios for the diffusivities of CO_2 and water in air for the pore and boundary layer respectively, i.e.

$$\frac{1}{g_{tc}} = \frac{1.60}{g_s} + \frac{1.37}{g_a} \qquad 2.14$$

Assuming that $C_o = C_a$ then

$$C_i = \frac{(g_{tc} - E / 2)C_o - A}{(g_{tc} + E / 2)} \qquad 2.15$$

The assumptions made in deriving this equation have recently been justified by direct measurement of C_i (Sharkey *et al*, 1982).

For simplicity the 'Jarman' correction to g_s and C_i has been ignored in some of the descriptive models and data transformations described in later chapters. The 'Jarman' correction used above only results in a correction for g_s of at maximum 3% for the experiments reported here.

2.6 Error analysis

Estimation of the likely errors involved in the determination of the variables measured using the gas analysis equipment is a very complex task because determinations of both E and A are based on differential

measurements and the size of these differentials are dependent on the flow rate of air through the assimilation chamber.

Ng (1978) made an attempt to estimate the error in the measurement of g_s using estimates of error for each of the individual measurements, i.e. measurement of flow, water vapour, leaf temperature, leaf area and boundary layer conductance. He applied a technique of root mean square error analysis. As pointed out by Morison (1980), the analysis was slightly pessimistic as measurements of water vapour pressure in the reference and sample lines are not totally independent, as Ng assumed, as a 'zero' measurement was made by passing the reference gas over both sensors immediately prior to the differential being measured. However, Ng only chose to give examples of errors for

a) a small value of D with a large value of g_s; error = ±8.4%

b) a large value of D with a small value of g_s; error = ±15.5%

These values do not cover cases where both D and g_s are small, as might be found when the stomata are essentially closed because of low leaf water potentials (see Chapter 8).

Much of the error in determining g_s can be attributed to the measurement of $(w_o - w_e)$. This error can be attributed to problems of zero drift and the repeatability of the instruments used to measure water vapour pressure. Thus this error will be absolute rather than proportional to g_s. Even with regular 'zero' readings it was found in practise that over half of the error in the system could be traced to this measurement.

An informative, though rather pessimistic exercise is to assume that the absolute error could account for ±5% of a large conductance i.e. ≈ 60% of Ng's ±8% estimate for g_s = 0.19 mol m^{-2} s^{-1}. If this error is absolute, the same error will apply to smaller conductances. Thus for a value of g_s of only 5% of 0.19 mol m^{-2} s^{-1}, i.e. 8 mmol m^{-2} s^{-1}, the error could be as high as ±100%, though this is likely to be the worse case.

Thus the mean square error for g_s, in percentage terms could possibly range from ±5 to ±100% depending on the absolute value of g_s. However, in most cases one is looking at the effect of a treatment on g_s relative to the control conditions for which g_s is large. In such cases an error of ±5% of the maximum value of g_s is acceptable compared to plant to plant variation of up to ±50%. Measurement errors are likely to be much more significant when one is trying to study changes in g_s when g_s is small, e.g. studying the response to D at low water potentials.

Neither Ng (1978) or Morison (1980) presented any quantitative estimates of errors for A, though Morison gave an estimate for the calibration of the absolute accuracy of the sensitivity of the infra-red gas analyser of ±5%. Reference to Janáč (1970) shows that the errors are likely to be similar, in percentage terms, to the errors in g_s, with the same problem of increasingly large percentage errors for smaller values of A, when the difference between the CO_2 concentrations in the air entering and leaving the chamber becomes small.

Estimation of error in determining C_i is more complex as C_i is calculated from estimates of g_s and A. Morison (1980) showed that errors in measuring flow and leaf area are not important as they are eliminated from the calculation of C_i. However, the largest sources of error, i.e. determination of $(C_e - C_0)$ and $(w_0 - w_e)$ are present as a product. Assuming, for large values of g_s and A that the absolute errors for both differentials is ±5%, then the mean square error for C_i would be approximately ±7%. However, if g_s and A are small, e.g. due to the effects of low water potential, and if both g_s and A are reduced to only 10% of the 'large' values but with the same absolute errors, g_s and A might each be subject to ±50% errors and estimation of C_i to a mean square error of *ca* ±70%. This was borne out in practice, as when g_s and A were small the calculated value of C_i sometimes came out as a negative value! Critical analyses of such data is clearly impossible and has been avoided in this thesis, though such errors often appear to be overlooked in many similar studies in the literature.

Similar arguments also apply to the estimation of the ratio of E/A.

The procedure outlined by Morison (1980) of minimising error by maintaining a reasonable difference in both CO_2 and H_2O mole fractions between the reference and sample lines was followed, i.e. to use as much plant material as possible, with low flow rates of air entering the chamber. Care was taken to prevent the CO_2 in the chamber dropping more than 25 μmol mol^{-1} below the mole fraction entering the chamber.

2.7 Data analysis and presentation

2.7.1 Standardisation

As shoot to shoot variation is often large in conifers, previous workers have normalised data when looking at the shape of response curves e.g Ng (1978), Morison (1980). The process of normalisation that they used was to first define a reference treatment, e.g. the lowest value of D for a g_s/D curve, or the highest light level for a g_s/light curve. A scaling factor, for each replicate, was then calculated to bring the actual values of the dependent variable to unity at the reference treatment. These scaling factors were then applied to the data for all treatments so that the shape of the response for each replicate was referenced to unity at the reference treatment.

This technique was applied to all the data presented in this thesis, except that, to allow absolute comparisons with other experiments, the data for each replicate was scaled to the actual, mean value of the dependent variable (for all replicates) at the reference treatment, rather than to a value of unity. This process is henceforth referred to as standardisation to distinquish it from normalisation.

As this process is similar to taking a percentage, corrections should be applied to statistical tests applied to such data e.g. an arcsine transformation for standard errors. However, as the application of conventional statistics to data based on only three or four replicates is borderline, the complication of applying such corrections was not considered justified. Therefore all statistics applied to the data assume a

normal distribution and such analyses must be considered as guides to trends in the data rather than critical tests.

In particular several experiments required that an attempt be made to seperate out the effects of two variables on the responses of the stomata e.g. light and leaf-to-air vapour pressure difference. In such cases an analysis of variance was applied to the standardised data. It is recognised that such analyses are of dubious validity. In addition, for some experiments, the measurements of g_s and A ranged from the light-saturated values to values close to zero. It is unlikely, for such data, that the assumption that all the treatments have equal variances will be true. Furthermore as the experiments were done in a predefined sequence of treatments, the treatments are also not random as assumed in such analyses.

2.7.2 **Curve fitting**

For most of the data presented in this thesis some form of curve fitting technique has been applied. For linear-regressions a local computer package called 'Presto' was used (see Appendix 4). For non-linear analyses the BMDP, PAR program for non-linear, least-squares analysis was used.

Assessing the goodness of fit of data by a model, and assigning estimates of error to the parameters derived by the analysis can be difficult (Ross, 1981). As advised by Ross, when comparing the fit of different models to the same data, the mean square error was used as a quantitative guide. No attempts at statistical comparisons of parameters are performed in this thesis, as this is an area of undefined statistics for non-linear models of this kind (Ross, 1981). However, to give some estimate of the likely error, the asymtopic standard deviations of any fitted parameters are given.

2.7.3 **Graphical presentation**

For the majority of experiments presented in this thesis, D was one of the treatment variables. D was imposed in steps, but from experiment to experiment it was not always possible to repeat exactly the absolute value of D at each step. Thus each level of D imposed has a margin of variation associated with it, e.g. see table 3.1. It was decided not to show these variations in the form of error bars on the graphs as, in general, the standard error was not much larger than the symbols for the points.

Where fitted curves are given with data points representing the means of several replicates, the likely variation in D must be be borne in mind when considering the fit of the curve.

As many of the graphs show several curves representing several treatments or species, for the sake of clarity only one standard error is plotted with each data point.

CHAPTER 3

THE RESPONSES TO LEAF-TO-AIR VAPOUR PRESSURE DIFFERENCE OF A RANGE OF CONIFEROUS SPECIES

3.1 Introduction

Papers presenting data, for conifers, that can be interpreted as showing a stomatal response to D, can be found dating back to 1964. Gindel (1964) presented field data for Aleppo pine of E as a function of 'evaporation intensity' (a measure of potential evaporation derived using an evaporimeter). These data showed a trend of declining E as 'evaporation intensity' increased. Gindel (1967) presented further field data of E for the same species, but with measurements of windspeed and relative humidity as well as 'evaporation intensity'. Again the data showed, in retrospect, some evidence for stomatal closure at low humdities but no detailed analysis of the data was performed, or discussion of the possible involvement of the stomata presented.

Whiteman & Koller (1964) presented both E and total shoot resistance to water loss (r_w) as a function of D, for potted Aleppo pine plants. As D increased r_w was found to increase markedly. As a result E increased linearly to a maximum (at a value of D of 1.67 kPa) and remained constant with further increases in D. However the authors discussed the increase in r_w in terms of incipient drying of the mesophyll, and did not consider a role for the stomata.

Similarly Hodges (1967) performed a survey of six species of conifers (Douglas-fir, ponderosa pine, western hemlock, grand fir, noble fir and Sitka spruce) in a range of conditions both in the field and in the laboratory. He found that in all the species A decreased, by varying degrees, during the middle of the day in the field. He was unable to explain this response in terms of light or temperature and thus looked at stomatal responses. He found that the stomata were closing at the same time and could account for a large part of the decrease in A. He correlated this closure with variation in D. However, both the field data and laboratory studies showed relatively large declines in leaf water

potentials concurrent with the stomatal closure. Thus he explained the stomatal closure as a response to the change in water potential which is linked to increased E driven by the increase in D; again the possiblity that there might be a direct response by the stomata to D is not considered.

Work on other species in the late 1960's and early 1970's led to the possibilty of either indirect responses of the stomata to D, via changes in leaf water potential e.g. Raschke & Kuhl (1969), or directly, via 'peristomatal transpiration' e.g. Lange *et al* (1971). This induced workers studying conifers to examine further the sensitivity of stomata to changes in D.

A number of papers published in the mid-seventies presented data which showed a range of stomatal responses in several different species to changes in D. Some of these data were field measurements of daily trends in g_s e.g. Fetcher (1976) (lodgepole pine), Running (1976) (Douglas-fir, ponderosa pine, western hemlock, grand fir, noble fir and Sitka spruce). The response of the stomata to environmental variables was qualitatively assessed from the diurnal trends.

Other workers have published data based on micrometeorological studies of canopy conductance. Generally the data analysis with regards to stomatal responses is crude, but Calder (1977) (Norway spruce), Roberts (1976) (Scots pine), Roberts (1978) (Norway spruce), Roberts (1983) (Scots pine) and Stewart & de Bruin (1984) (Scots pine) all showed that the species studied closed their stomata as D increased.

A larger proportion of the data presented in the literature consisted of field measurements with some quantitative extraction of the response of g_s to D, e.g. Neilson & Jarvis (1975) (Sitka spruce), Watts *et al* (1976) (Sitka spruce), Tan & Black (1976) (Douglas-fir), Kaufmann (1976) (Engelmann spruce), Tan *et al* (1977) (Douglas-fir), Running (1980) (lodgepole pine), Benecke *et al* (1981) (European larch) and Leverenz (1981a+b) (Douglas-fir). However, in these papers either all the environmental variables (in particular plant water status and temperature) were not measured thoroughly, or the data extraction techniques only removed the effects of one of the factors that are now considered to interact with D. For

example Watts *et al* (1976) discussed the problems of separating the respone of g_s to D and T for their field data. However, all these studies showed, qualitatively that the stomata of all the species studied did close, to varying degrees, as D increased.

In some field studies e.g. Rutter (1978) (ponderosa pine, white fir and incense-cedar) more rigorous techniques were applied to analyse the data. Rutter's data showed that the stomata of all the three species he studied exhibited a strong response to D. Rutter ranked the species as follows: incense-cedar with the strongest response (at 4.7 kPa the stomata being closed to only 20% of their conductance at 0.5 kPa), followed by white-fir, then ponderosa pine. For all species the shape of the response curves was that of an exponential decline of g_s with increasing D i.e. at low D (large g_s) g_s declined rapidly, but as D increased the rate of closure decreased. This type of stomatal response leads to an hyperbolic relationship between E and D, with E approaching an asymptote at large values of D. Thus his data lend no support to the possibilty that the stomata have a 'direct' response to D in these species. Unfortunately though, Rutter did not also consider the influence of temperature or plant water status and it can be argued that these variables may have influenced the responses he derived.

For more critical analyses of the response of the stomata of conifers to D one must turn to experiments done in controlled environments where other dependent variables can be controlled more easily.

Grace *et al* (1975) and Watts & Neilson (1978) presented data for Sitka spruce seedlings which showed similar marked response of g_s to D. The responses were very similar to those described above for the work of Rutter i.e. the stomata closed so that E reached an asymptote as D increased. Bennett & Rook (1978) found a similar response for two clones of radiata pine. They found that the stomata maintained E at an almost constant value over the limited range (0.3 - 1.4 kPa) of D that they studied. Meizner (1982) also reported similar responses for Douglas-fir and, in addition, showed that the strength of response varied with the age of the needles. Current year shoots were found to have a comparatively strong response of g_s to D (E reached an asymptote, and possibly declined

slightly at higher values of D) whilst the shoots of the previous season had a weaker response (E continued to increase with D).

In contrast Kaufmann (1976) showed an extremely strong stomatal response to D in laboratory experiments on Engelmann spruce. However, several questions have to be asked regarding the methodology of these experiments. The results contrast markedly to the field data he also presented in the paper, both in the strength of response (the stomata closed to only 25% of their conductance at 0.4 kPa, when D was increased to 0.9 kPa) and also in the absolute magnitude of the reported conductances (the maximum value shown being only 0.016 mol m^{-2} s^{-1} at 0.4 kPa and 420 µmol m^{-2} s^{-1} photon flux density). He also did an experiment to compare the effect of water potential on the response of g_s to D. Although this showed that the stomata of the plants in the stress treatment, in general had lower conductances, some of the data appeared to show that plants with xylem water potentials less than -1.5 MPa opened their stomata when D was low to the same degree as plants which were unstressed. This finding conflicts with data presented later in this thesis and with that of other workers who have studied the interactions between D and water stress in conifers e.g. Tan *et al* (1977), and other species, e.g. Schulze & Kuppers (1979).

The most likely reason for these anomalies is the technique used to measure g_s. Kaufmann used a transit-time porometer with which problems of calibration were encountered. In a later paper by the same group (Kaufmann & Eckard, 1977) a more advanced version of this instrument with an improved chamber and humidity sensor is described. This instrument, despite improvements in design, was found to require complex empirical corrections to account for the past history of temperature and humidity that the porometer had experienced. Such corrections were found to be particularly essential when the humidity was changed because of surface adsorption and release of water vapour in the chamber (see also Gandar & Tanner, 1976; Hack, 1980). Errors induced by ignoring these factors were also largest at high humidities i.e. when D was small. Thus the measurements presented in Kaufmann (1976) at low D's (where the stomata appear to open), using the old design of porometer, without such corrections may well be subject to extremely large errors.

Similar methodological problems may also explain some of the results reported by Johnson & Ferrell (1983) for Engelmann spruce and Douglas-fir measured in a growth room with a null-balance, steady-state porometer. The data showed that the stomata closed with increasing D when grown at 35 °C but not at 20 °C. However, they described a 'chamber effect' of repeated measurements which they attributed to a plant response. The speed at which they make their measurements (within 30 to 120 s) is more likely to have been the cause of such effects. The null-balance porometer can also suffer from absorption and release of water vapour from the chamber walls, particularly when the balance-point humidity has been changed. A large step in humidity can have a carry-over effect on the measurements of g_s for tens of minutes, even with a well-designed cuvette (see Chapter 4). Thus the apparent strength of response at 35 °C and lack of response at 20 °C must be treated with caution.

Perhaps the most striking response of g_s to D that has been reported for a conifer is that of Ng (1978) for Scots pine. Using an open-gas exchange system, taking steady-state measurements he reported a stomatal closure which, at 20 °C, resulted in E rising to a peak as D was increased from 0.4 to 1.2 kPa and then rapidly declining to only 20% of its peak value when D was 1.8 kPa. Furthermore at 10 °C E declined continually as D was increased from 0.4 to 0.9 kPa. Such a result clearly requires a direct mechanism of stomatal response to D, as proposed by Farquhar (1978).

However, Ng (1978) also presented the results of two further experiments on similar Scots pine plants. The experiments were done in a wind-tunnel with daily, stepped changes in D, using a null-balance porometer for measurement. At 23 °C the stomata closed by *ca* 55% as D was increased from 0.4 to 1.6 kPa. This closure, if replotted does not result in E declining as D was increased. At 15 °C the stomata closed from by *ca* 50% as D was increased from 0.1 to 1.05 kPa; again this does not result in a decline in E as D is increased. Furthermore cut-shoots with very high water potentials showed no significant response to D, thus this data showed no requirement for a 'direct' response of the stomata to D.

Although the stomatal mechansim by which the stomata of conifers respond to humidity is still in question the fact that the stomata generally close to some degree as D increases is, however, well established. This closure inevitably results in some reduction of A, particularly during the middle of the day when conditions may be otherwise ideal for A (Hodges, 1967). The number of reports of concurrent measurements of A and g_s in response to D are however comparatively few . In particular it is unfortunate that Ng (1978) did not present concurrent measurements of A for the very strong response of g_s to D.

Whiteman & Koller (1964) found for Aleppo pine that A declined linearly as D increased. The decline in A appeared to precede an increase in stomatal resistance. Hodges (1967) found for noble and grand fir that initially as relative humidity was decreased A remained constant. Then, after a step from 75% to 45% relative humidity (T not specified) A started to decline. In the case of grand fir this decline preceded any detectable closure of the stomata.

Grace *et al* (1975) did not measure A directly, but reported a decline in growth rate for Sitka spruce plants grown at a higher D compared to a control.

Watts & Neilson (1978) presented measurements for Sitka spruce of gross A ($^{14}CO_2$ uptake) as D was varied, in daily steps. They showed only a slight (10%) decline in A as D was increased from 0.05 to 1.5 kPa (g_s declined by 60%), then a sudden decline of *ca* 30% as D was increased to 1.8 kPa (g_s declining only by 10% over this range).

In contrast Benecke (1980) presented field data for radiata pine showing a linear decline in A of 30% as D increased from 0.7 to 1.8 kPa. Similarly Meizner (1982) showed that shoots of Douglas-fir showed an almost linear decline in A of 50% as D increased from 0.5 kPa to 1.8 kPa.

Thus the literature presents a wide range of responses of both g_s and A in response to changes in D. Whether or not these differences are due to differences amongst species, growth conditions or measurement

techniques is hard to establish.

It seemed necessary, therefore, to make a comparison of a limited range of conifers under controlled laboratory conditions, trying to avoid some of the pitfalls of methodological approach discussed above. This was done particularly to see if the responses described by Ng (1978) could be reproduced in other species, especially those important in British commercial forestry. To allow comparison with Ng's data every attempt was made to use an experimental procedure identical to that which he used.

3.2 **Plant material**

Measurements were made on Sitka spruce (Queen Charlotte Islands provenance No.1004), lodgepole pine (provenance Terrace, B.C. No. 7114), hybrid larch (provenance Laigh of Moray, No. NT8) and Scots pine (provenance NT 10). The plants were all (1+2)-year-old potted seedlings grown for the last year in U.C. 2Cd peat-based soil mix (Matkin & Chandler, 1957). All plant material originated from the Forestry Commission Northern Research Station, The Bush Estate, Midlothian.

Because of supply problems the shoots of the different species were not of the same age. Data for two age classes of Scots pine shoots are presented:

i) shoots which were only 8 weeks old since bud break (henceforth called new Scots pine). Plants at the end of their 2nd year in pots, were brought into the preconditioning growth rooms in early May. The higher temperatures and prolonged daylength (see below) caused the shoots to break bud immediately. The plants remained in these conditions until the start of the experiment, 8 weeks after they were brought inside.

ii) shoots which were ten months old (called old Scots pine). The plants had broken bud the previous year (at the beginning of their 2nd year in pots) outside, under 'natural conditions'. In early March

they were brought into the growth rooms. After three weeks the experiments were done. There were no visible signs of bud break for the shoots used in the experiment.

For Sitka spruce, lodgepole and hybrid larch the plants broke bud under natural conditions, outside, at the end of their 2nd year in pots. The Sitka spruce and lodgepole pine were brought into the growth rooms nine weeks after breaking bud, three weeks prior to the experiment. Thus the shoots were 12 weeks old at the start of the experiment.

The hybrid larch were brought in 13 weeks after breaking bud, three weeks prior to the experiment. These shoots were 16 weeks old at the start of the experiment.

The growth room conditions for all treatments was an average of 20 ^{o}C (day and night), 75% relative humidity (<=> to an air saturated vapour pressure deficit of 0.58 kPa) with a daylength of 16 h.

Current year shoots from the first whorl were measured in all instances. The shoots for each species were done on sequential days.

3.3 **Experimental details**

When the plants were initially brought into the growth room the shoots to be measured were selected. Needles were removed from a length of the stem to allow insertion through the seal in the assimilation chamber. Thus the plants had several weeks to recover prior to the experiment. The plants were watered every other day to pot capacity.

The evening prior to the experiment the plant to be measured was watered well and placed in an opaque, black plastic bag. The shoot which was to be measured protruded through a hole in the side of the bag. The bag was used to minimise transpiration from the bulk of the plant, thus minimising changes in the bulk water potential of the plant. The shoot was then inserted into the assimilation chamber and the gas exchange system set in operation. The conditions in the chamber were

darkness, a leaf temperature of 20 °C with D set to 1.0 kPa. The flow of air into the chamber was set to a rate much higher than used in the experiment so that any changes in rate of transpiration by the shoot would have little effect on D in the chamber when the system was left unattended overnight. D was set at 1.0 kPa, rather than the growth room condition, as this minimised the risk of condensation in the chamber when the lights were switched on the next morning.

On the day of the experiment the lights were switched on automatically at the same time as those in the growth room. When the temperature control system had settled to compensate for the added heat load of the lamps, the flow rate of air into the chamber was reduced to the rate used for measurement and D was adjusted to the starting condition of *ca* 0.4 kPa. For all species D was increased from *ca* 0.4 to *ca* 1.9 kPa in five steps, with an equilibration time of 100 minutes for each step.

Prior to the start of measurements the chamber was briefly opened and three fascicles were removed for determination of water potential. Preliminary experiments had shown that removal of fascicles did not present any significant sources of transpiration from the broken tissue - the surfaces become covered with resin within a few minutes. At the end of the experiment three further fascicles were removed from the shoot being measured, plus another three from a similar shoot inside the plastic bag. All the needles were then removed from the shoot that had been measured for plan leaf area determination.

To summarise, for each type of plant material there were three replicates (3 shoots on different plants). In each experiment there were six D treatments which were imposed by increasing D. For each replicate in each experiment both E and A were measured. For each replicate there are also three sets of water potential measurements.

3.4 Results

The data was standardised using the procedure outlined in Chapter 2. The scaling values for each replicate were the same for both g_s and E. A was standardised independently, but also relative to the mean of the three replicates at the lowest D. The results of g_s, E and A to D are presented for all four species in figures 3.1, 3.2 and 3.3 respectively. A summary of the plotted data and the mean values of the unstandardised i.e. raw data are given in table 3.1.

The fitted curves shown in the figures were derived in the case of:

i) E by fitting a rectangular hyperbola to standardised data for all three replicates using a non-linear least squares analysis (see Appendix 4 for details). A linear, a non-rectangular, a 2nd-order quadratic and a 'natural growth' function (Parton & Innis, 1972) were also fitted, but the rectangular hyperbola of the form:

$$E = \frac{E_m \; a \; D}{E_m + a \; D} \qquad 3.1$$

was found to give the smallest mean square error in the majority of cases. The units and parameters derived from this equation are given in table 3.2. The only data which are clearly not fitted well by this function are those for the new Scots pine, but it was decided to use the same function for all the data.

ii) g_s by solving the relationship fitted for E to give g_s as follows:

using $$g_s = \frac{E \; P}{1000 \; D} \qquad 3.2$$

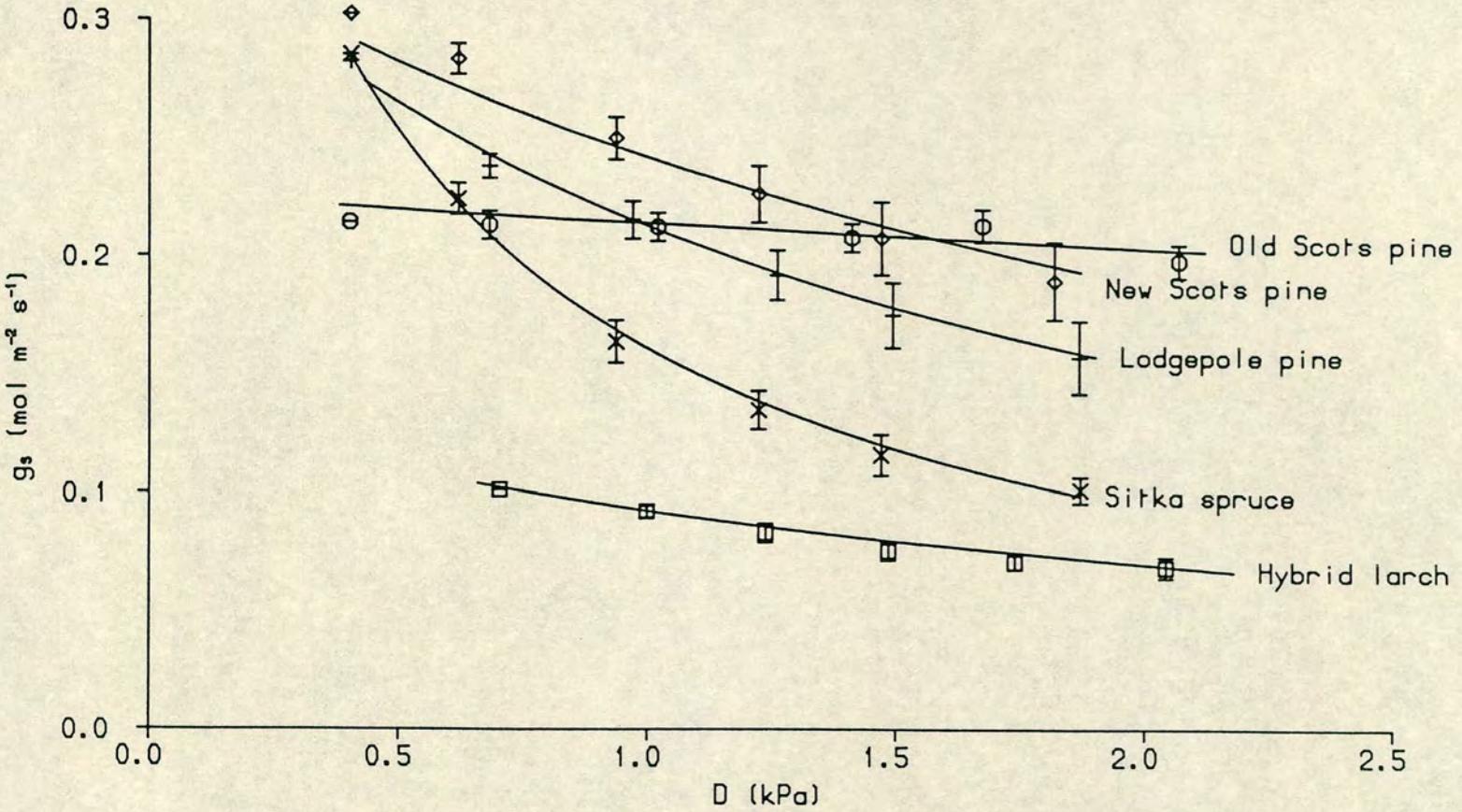

Figure 3.1: g_s as a function of D for a range of species. Data points represent the mean of 3 replicates, plus 1 S.E. See the text for a description of the fitted curves.

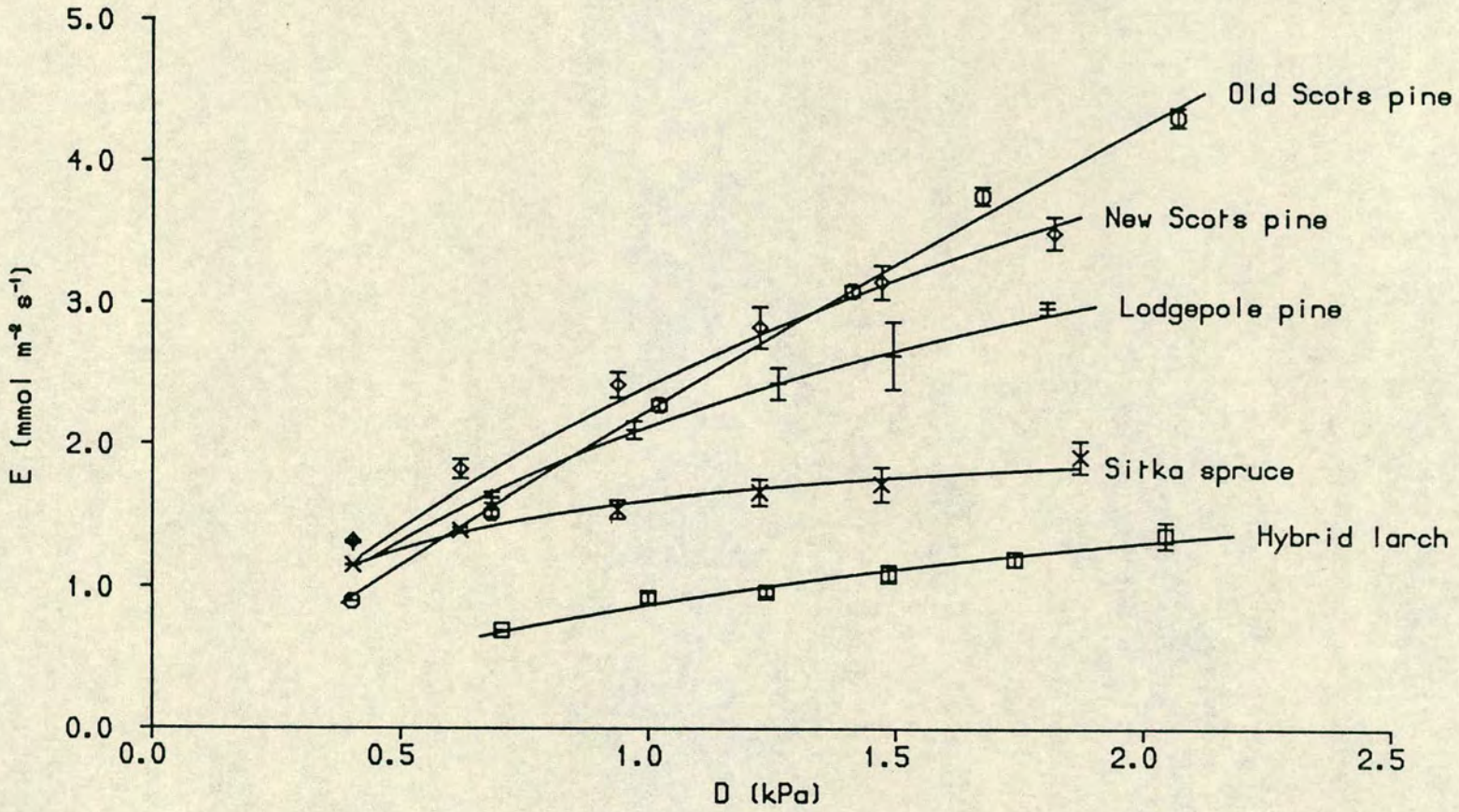

Figure 3.2: E as a function of D for a range of species. Data points represent the mean of 3 replicates, plus 1 S.E. See the text for a description of the fitted curves.

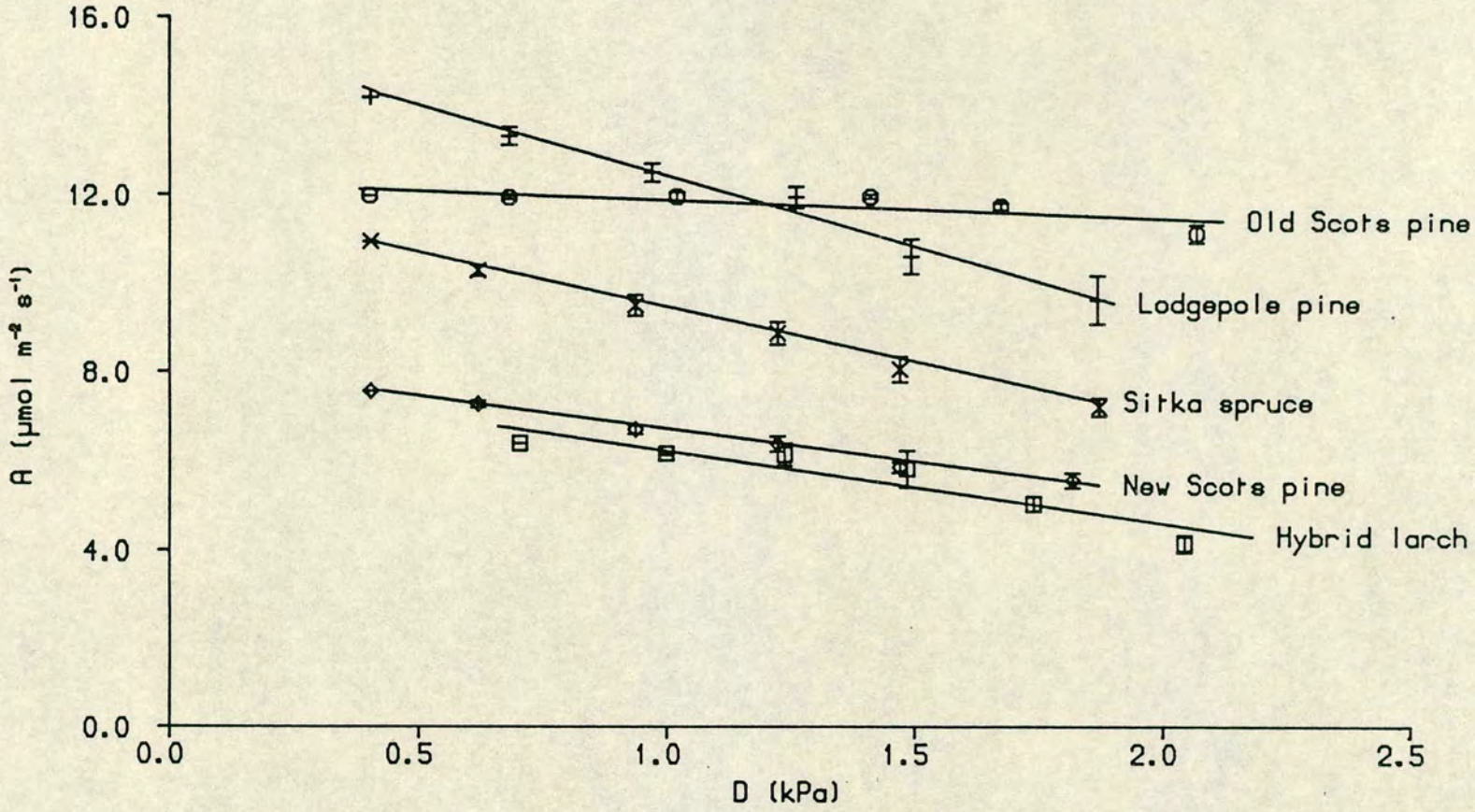

Figure 3.3: A as a function of D for a range of species. Data points represent the mean of 3 replicates, plus 1 S.E. The lines were fitted by linear regressions.

Table 3.1 A summary of the raw data for the species comparisons shown in figures 3.1, 3.2 and 3.3. The data for three replicates has been independently standardised to the mean values for conductance, transpiration and assimilation at the lowest value of D for each experiment. The values given are the means (with one standard errors in brackets) of three replicates for each level of D.

i) Sitka spruce
At the lowest D (0.40 kPa) the unstandardised mean of -

a) g_s was 0.285 (±0.053) mol m^{-2} s^{-1}
b) E was 1.152 (±0.211) mmol m^{-2} s^{-1}
c) A was 10.93 (±1.483) µmol m^{-2} s^{-1}

Standardised data (units as above):

g_s	E	A	D
0.2850 (±*****)	1.152 (±*****)	10.93 (±*****)	0.403 (±0.003)
0.2243 (±0.013)	1.398 (±0.036)	10.28 (±0.256)	0.621 (±0.020)
0.1637 (±0.018)	1.544 (±0.130)	9.45 (±0.477)	0.937 (±0.020)
0.1347 (±0.016)	1.665 (±0.180)	8.86 (±0.504)	1.226 (±0.012)
0.1157 (±0.017)	1.723 (±0.239)	8.05 (±0.573)	1.473 (±0.011)
0.1010 (±0.011)	1.916 (±0.228)	7.22 (±0.411)	1.870 (±0.001)

ii) Lodgepole pine
At the lowest D (0.41 kPa) the unstandardised mean of -

a) g_s was 0.282 (±0.034) mol m^{-2} s^{-1}
b) E was 1.306 (±0.139) mmol m^{-2} s^{-1}
c) A was 14.19 (±1.89) µmol m^{-2} s^{-1}

Standardised data (units as above):

g_s	E	A	D
0.2820 (±*****)	1.306 (±*****)	14.19 (±*****)	0.406 (±0.014)
0.2380 (±0.010)	1.624 (±0.074)	13.32 (±0.401)	0.683 (±0.006)
0.2153 (±0.016)	2.101 (±0.127)	12.49 (±0.410)	0.970 (±0.011)
0.1920 (±0.021)	2.432 (±0.220)	11.93 (±0.493)	1.260 (±0.020)
0.1750 (±0.028)	2.632 (±0.417)	10.59 (±0.778)	1.493 (±0.009)
0.1570 (±0.031)	2.971 (±0.612)	9.61 (±1.093)	1.867 (±0.028)

iii) Hybrid larch
At the lowest D (0.70 kPa) the unstandardised mean of -

a) g_s was 0.101 (±0.008) mol m^{-2} s^{-1}
b) E was 0.695 (±0.056) mmol m^{-2} s^{-1}
c) A was 6.39 (±0.30) µmol m^{-2} s^{-1}

Standardised data (units as above):

g_s	E	A	D
0.1010 (±*****)	0.695 (±*****)	6.39 (±*****)	0.704 (±0.024)
0.0920 (±0.005)	0.925 (±0.076)	6.17 (±0.081)	0.999 (±0.006)
0.0830 (±0.008)	0.966 (±0.057)	6.15 (±0.495)	1.237 (±0.015)
0.0753 (±0.008)	1.096 (±0.122)	5.82 (±0.829)	1.485 (±0.005)
0.0703 (±0.006)	1.201 (±0.103)	5.04 (±0.333)	1.739 (±0.003)
0.0683 (±0.009)	1.371 (±0.184)	4.15 (±0.398)	2.043 (±0.071)

iv) New scots pine
At the lowest D (0.40 kPa) the unstandardised mean of -

a) g_s was 0.302 (±0.022) mol m^{-2} s^{-1}
b) E was 1.320 (±0.090) mmol m^{-2} s^{-1}
c) A was 7.55 (±0.59) µmol m^{-2} s^{-1}

Standardised data (units as above):

g_s	E	A	D
0.3020 (±*****)	1.320 (±*****)	7.55 (±*****)	0.403 (±0.014)
0.2830 (±0.013)	1.824 (±0.135)	7.27 (±0.156)	0.620 (±0.026)
0.2497 (±0.018)	2.423 (±0.180)	6.70 (±0.206)	0.936 (±0.020)
0.2263 (±0.024)	2.830 (±0.295)	6.38 (±0.338)	1.223 (±0.005)
0.2077 (±0.031)	3.152 (±0.239)	5.86 (±0.237)	1.470 (±0.006)
0.1897 (±0.033)	3.503 (±0.228)	5.57 (±0.334)	1.817 (±0.054)

v) Old scots pine
At the lowest D (0.40 kPa) the unstandardised mean of -

a) g_s was 0.214 (±0.025) mol m^{-2} s^{-1}
b) E was 0.899 (±0.100) mmol m^{-2} s^{-1}
c) A was 11.97 (±0.68) µmol m^{-2} s^{-1}

Standardised data (units as above):

g_s	E	A	D
0.2142 (±*****)	0.899 (±*****)	11.97 (±*****)	0.401 (±0.009)
0.2132 (±0.006)	1.516 (±0.025)	11.93 (±0.041)	0.683 (±0.021)
0.2123 (±0.006)	2.279 (±0.040)	11.94 (±0.110)	1.020 (±0.020)
0.2080 (±0.006)	3.089 (±0.040)	11.94 (±0.046)	1.410 (±0.000)
0.2130 (±0.007)	3.763 (±0.064)	11.72 (±0.076)	1.673 (±0.011)
0.1980 (±0.007)	4.317 (±0.067)	11.10 (±0.199)	2.067 (±0.015)

***** - standard errors are not given as due to the process of standardisation they are forced to zero.

then

$$g_s = \frac{P E_m a}{1000(E_m + a D)} \qquad 3.3$$

Where units of g_s, E and D are as in table 3.1; units of E_m and a are given in table 3.2. and P is atmospheric pressure (kPa). The factor of 1000 is to correct for the difference in magnitude of the units of E, a and g_s.

It is interesting to note that although this relationship was defined with respect to E, the slope parameter (a) is related to the maximum conductance, at D=0, as follows:

$$g_{max} = \frac{P a}{1000} \qquad 3.4$$

iii) A by fitting a linear regression to the A *versus* D data. A linear fit was found to give the smallest residual sum of squares in all cases when compared to other curves. The derived parameters for the linear curve are shown in table 3.3.

Table 3.2 The parameters derived from fitting hyperbolic curves, of the form of equation 3.1, to the E *versus* D data for each species. The asymptotic standard deviations of the parameters are given in the brackets. Units for E_m are mmol m^{-2} s^{-1} and for a are mmol m^{-2} s^{-1} kPa^{-1}. N=18.

Species	E_m	a
Sitka spruce	2.210 (±0.245)	5.904 (±1.760)
Lodgepole pine	5.437 (±1.687)	3.490 (±0.899)
Hybrid larch	2.682 (±0.786)	1.301 (±0.278)
New Scots pine	8.469 (±3.531)	3.382 (±0.760)
Old Scots pine	42.30 (±20.94)	2.371 (±0.110)

Table 3.3 The parameters derived from a linear regression of A as a function of D. Standard errors are given in brackets. The units for the slope are μmol m^{-2} s^{-1} kPa^{-1} and for the intercept are as for A in table 3.2.

Species	Slope	Intercept	r^2
Sitka spruce	-2.568(±0.284)	11.93(±0.34)	0.8362
Lodgepole pine	-3.207(±0.490)	15.62(±0.60)	0.7277
Hybrid larch	-1.625(±0.389)	7.84(±0.56)	0.5217
New scots pine	-1.452(±0.207)	8.17(±0.25)	0.7546
Old scots pine	-0.427(±0.136)	12.29(±0.18)	0.3809

To test the significance of the effect of D on g_s and A for each species an analysis of variance was done, using the standardised data and treating each step in D as a different treatment. The limitations of the validity of such tests when using standardised data, as discussed in Chapter 2, should be born in mind when assessing the results of such analyses which are given in the two left hand columns of table 3.4.

Table 3.4 The percentage reduction of g_s and A caused by increasing D from the lowest to highest value during each experiment. Also given is the level of significance of the effect of D on g_s and A, for all values of D (as given by an F test). These values were determined by applying an analysis of variance to the standardised data for all three replicates. Steps of D, as in table 3.1, are taken as being different treatments.

Species	% Reduction in g_s	% Reduction in A	% sig. level of D on g_s.	% sig. level of D on A.
Sitka spruce	64.6	34.1	0.1	0.1
Lodgepole pine	44.3	32.3	5.0	1.0
Hybrid larch	32.4	37.1	5.0	5.0
New Scots pine	37.1	26.3	5.0	0.1
Old Scots pine	7.5	7.3	N.S.	5.0

N.S. - not significant at the 5% level.

The results show that for all but the old Scots pine plants there is a significant decline in stomatal conductance as D was increased. The

concurrent declines in A were all significant at the 5% level. The E/D curves in figure 3.2 show that for those species with a significant response of g_s to D, the stomatal closure results in E being non-linear, i.e. E increases less with D than it would if conductance was constant. In the case of Sitka spruce, which has the strongest response, E only increased by 40% (over the range of measurement) whilst D was increased by 370%.

The results of the measurements of water potential are presented in table 3.5. An analysis of variance was done using the data for each shoot to test if there were differences between the sets of water potential measurements. This analysis shows, for the shoots whose gas exchange was measured, that for all but one larch and one new Scots pine shoot there was a decline in xylem water potential (significant at the 5% level). The average decline for each species was, however, only in the range of -0.04 to -0.09 MPa.

The measurements for shoots, similar to those studied, showed that the water potential of these needles did not decline significantly when comparing measurements made at the start and end of each experiment. The water potentials and their changes appears to show few differences between species. Hybrid larch, for some unknown reason, had lower water potentials at the start of the day, than the other species.

As some previous workers present their data as stomatal resistances rather than conductances, the data is replotted in fig. 3.4. The fitted curves have been derived from the inverse of equation 3.3 using the same parameters as in table 3.2. Roberts (1983) proposed that the relationship between r_s and D may be linear and fairly constant for a range of conifers. The inverse of fitted equation 3.3 appears to support this as it is linear with respect to D. To test this further linear regressions were performed for the standardised r_s/D data, independently of the curve fitting of E/D. The parameters derived and the coefficients of multiple determination are shown in table 3.6. For all of the plant material measured a linear curve does fit the data well, but the slopes and intercepts vary considerably.

Table 3.5 A summary of the needle xylem water potential measurements. The means (in MPa) of 3 needles are presented (with standard error of the mean in brackets). Data are presented for measurements before and after the experiment for the shoot being measured, plus measurements for needles taken from shoots on the same whorl (but inside the black plastic bag). The results of a students T-test between the means for each shoot are given. The average difference between the shoots before and after the experiment have been calculated using data from all three replicates.

Species	Shoot	Before	After	Similar shoot,after	% Level of sig.diff. B-A	B-S	A-S
Sitka spruce	1	-0.59(±.01)	-0.64(±.01)	-0.62(±.01)	1.0	10	10
	2	-0.42(±.02)	-0.53(±.01)	-0.40(±.01)	0.1	10	0.1
	3	-0.47(±.01)	-0.55(±.02)	-0.35(±.02)	0.1	10	0.1
	Mean difference (Before-After) = -0.08 MPa						
Lodgepole pine	1	-0.41(±.01)	-0.51(±.03)	-0.38(±.02)	5.0	10	1.0
	2	-0.43(±.01)	-0.55(±.02)	-0.43(±.02)	1.0	10	1.0
	3	-0.50(±.01)	-0.55(±.01)	-0.52(±.01)	5.0	10	10
	Mean difference (Before-After) = -0.09 MPa						
Hybrid larch	1	-0.69(±.01)	-0.77(±.02)	-0.70(±.01)	5.0	10	5.0
	2	-0.69(±.02)	-0.70(±.02)	-0.69(±.03)	10	10	10
	3	-0.52(±.01)	-0.56(±.01)	-0.53(±.01)	5.0	10	10
	Mean difference (Before-After) = -0.04 MPa						
New Scots pine	1	-0.41(±.02)	-0.48(±.02)	-0.41(±.03)	10	10	10
	2	-0.36(±.03)	-0.47(±.02)	-0.39(±.01)	5.0	10	5.0
	3	-0.37(±.03)	-0.49(±.03)	-0.41(±.01)	1.0	10	5.0
	Mean difference (Before-After) = -0.07 MPa						
Old Scots pine	1	-0.49(±.01)	-0.58(±.02)	-0.50(±.01)	1.0	10	1.0
	2	-0.68(±.02)	-0.78(±.03)	-0.72(±.01)	5.0	10	10
	3	-0.60(±.02)	-0.67(±.00)	-0.62(±.01)	1.0	10	5.0
	Mean difference (Before-After) = -0.09 MPa						

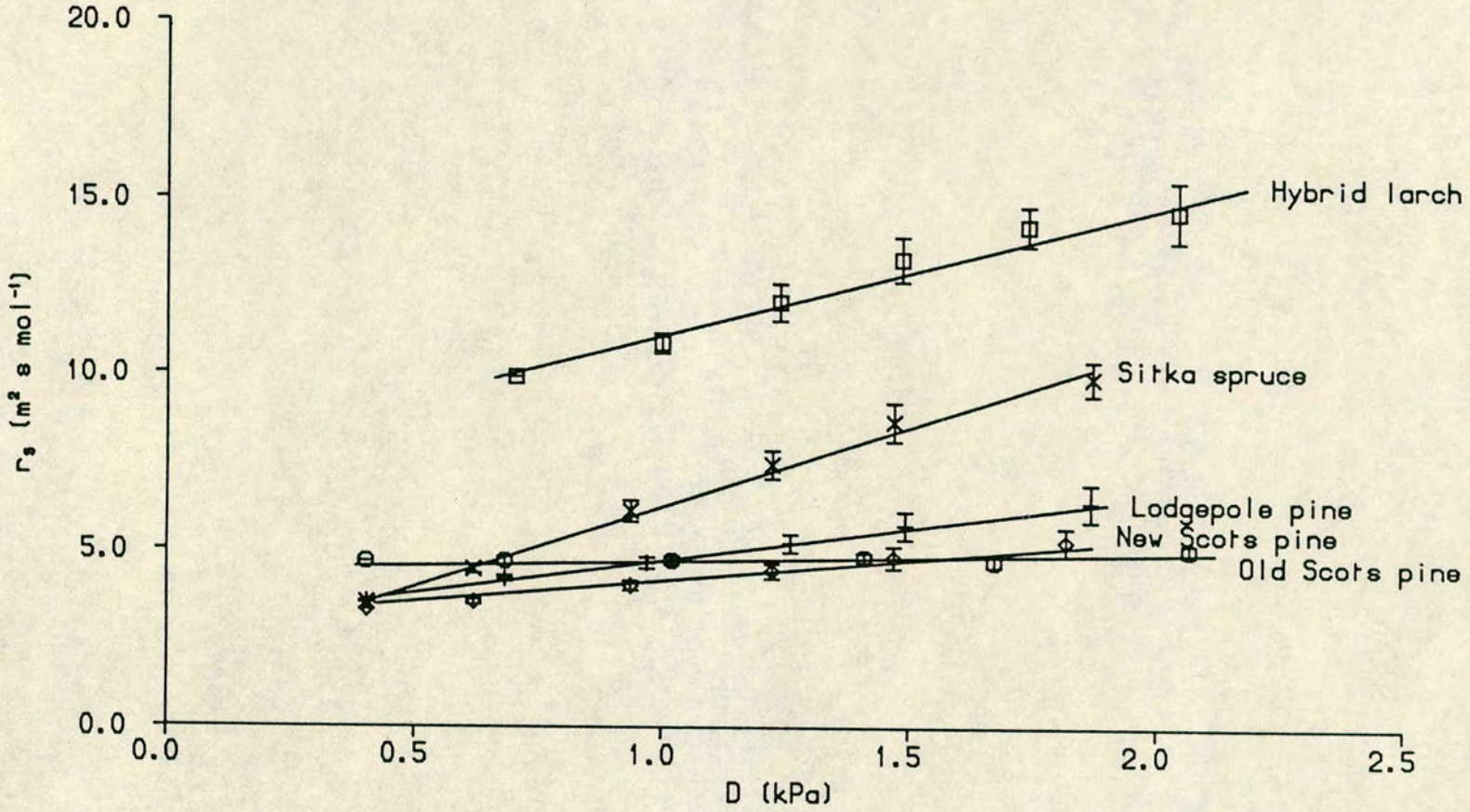

Figure 3.4: r_s as a function of D for a range of species. Data points represent the mean of 3 replicates, plus 1 S.E. See the text for a description of the fitted curves.

Table 3.6 The results of a linear regression of r_s on D. The standard errors of the parameters are given in brackets. The units for the slope are all in m^2 s mol^{-1} kPa^{-1}. The units for the intercept are m^2 s mol^{-1}.

a) For the data presented above.

Species	Slope	Intercept	r^2
Sitka spruce	4.485 (±0.184)	1.798 (±0.219)	0.9934
Lodgepole pine	1.912 (±0.044)	2.819 (±0.053)	0.9979
Hybrid larch	3.799 (±0.306)	7.293 (±0.441)	0.9746
New Scots pine	0.178 (±0.749)	4.555 (±0.100)	0.5857

b) For data presented in the literature. The data have been read from diagrams in the papers and approximate corrections applied to convert the units.

Source	Species	Slope	Intercept	r^2
Grace *et al* (1975) (fig. 4)	Sitka spruce	14.47 (±0.83)	3.77 (±0.56)	0.990
Watts & Neilson (1978) (fig. 3)	Sitka spruce	3.64 (±0.50)	3.52 (±0.51)	0.913
Bennett & Rook (1975) (fig. 2) * see note.	Radiata pine Clone 456.	33.48 (±4.41)	-4.61 (±4.25)	0.935
	Clone 457.	10.24 (±1.63)	-0.89 (±1.53)	0.908
Meizner (1982) (fig.6,plant 1)	Douglas-fir	21.66 (±2.59)	9.54 (±3.39)	0.972
Roberts (1983) (fig. 1a)	Scots pine	3.75 (±0.57)	0.88 (±0.49)	0.715

* converted to plan area, using their conversion factor.

c) Using models presented in the literature. Two of the references given by Roberts (1983) present a simple model of stomatal conductance as a function of D i.e.

$$g_s = g'_{max} (1 - kD)$$

g'_{max} is the maximum conductance i.e. under light saturated conditions.

This is non-linear for r_s as a function of D, but the non-linearity may be small. To test this, data were generated, using the models, for r_s in the range of D of 0.1 to 2.0 kPa in steps of 0.1 kPa. A linear regression was then applied to these data, to allow comparison with the data above. Units are as above.

Source	Species	Slope	Intercept	r^2
Jarvis (1976)	Sitka spruce	3.10 (±0.14)	5.11 (±0.17)	0.964
	Douglas-fir *	1.32 (±0.02)	8.20 (±0.03)	0.995
Calder (1977)	Norway spruce	5.95 (±0.96)	-1.03 (±1.15)	0.680

* Not presented by Roberts (1983).

As a first step in the analysis of the effect of changing D on the water loss/CO_2 uptake balance the ratio of E/A has been calculated. These values are shown in fig. 3.5. The fitted curves have been generated by using the rectangular hyperbolas fitted for E as a function of D divided by the linear curves for A as a function of D.

To show the sensitivity of the stomata to D the slope of the g_s/D curve was calculated by differentiating the fitted g_s/D function given above. This procedure assumes that all of E is under stomatal control, i.e. the cuticular component is small. Although no attempt was made to measure cuticular tranpiration directly, measurements of 'predawn' conductances resulted in values less than 5%, of the maximum measured under high light levels, in all species. As a large part of this may result from dark stomatal opening the cuticular component was considered to be insignificant. The function used was thus:

$$\frac{dg_s}{dD} = \frac{-P E_m a^2}{1000(E_m + a D)^2} \qquad 3.5$$

The plots of this function for the different species are given in fig. 3.6. In addition, to test the hypothesis of Morison & Gifford (1983) that the sensitivity of stomata may be species independent and correlated to the absolute stomatal conductance, dg_s/dD is also plotted against D in fig. 3.6. As the curves plotted in both figures 3.5 and 3.6 have been calculated from fitted curves no data points or estimate of error can easily be attached. The significance of the shape of these curves must therefore be treated with caution.

3.5 Discussion

The response of the stomata to D showed considerable variation both amongst the different species studied and also between the two ages of shoot of the Scots pine. The conclusion that older shoots respond less to D requires further testing but this finding is in broad agreement with the

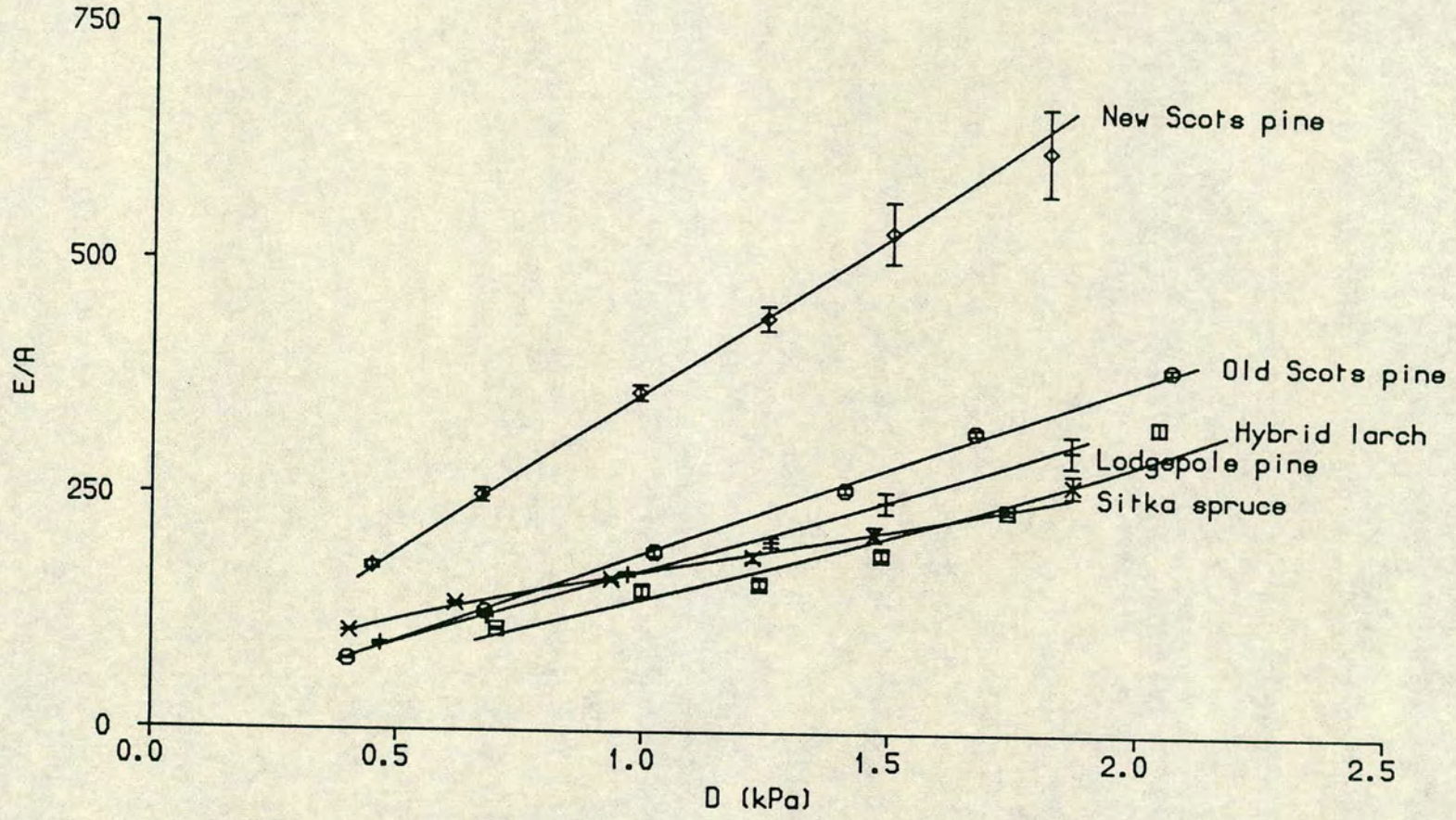

Figure 3.5: E/A as a function of D for a range of species. Data points represent the mean of 3 replicates, plus 1 S.E. See the text for a description of the fitted curves.

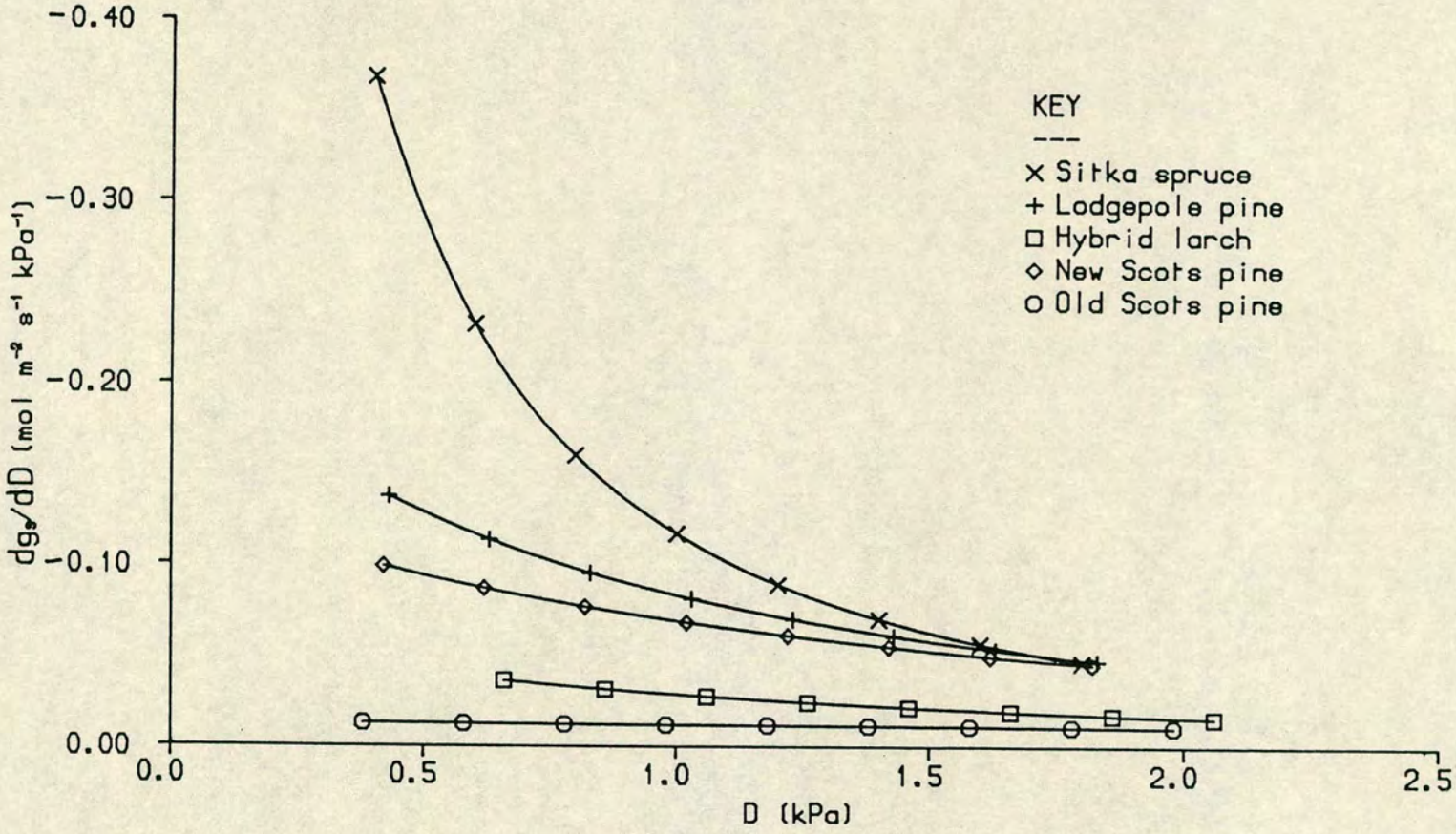

Figure 3.6: dg_s/dD as a function of D, for a range of species. The curves represent transformations of the functions fitted to the E *versus* D data.

results of Neilson & Jarvis (1975) and Meizner (1982). The contrast in the response of the two shoot ages shows that inter-specific comparisons are difficult to make as such variation may also be found in the other species and possibly between different provenances. Seasonal variation in the absolute value of stomatal conductance (see Neilson & Jarvis, 1975) also complicates such comparisons.

In none of the species/experiments was there a decline in E as D was increased. The hyperbolic function which fitted the E/D data best shows this clearly as E approaches an asymptote as D gets larger. The lack of a decline in E at large D is in marked contrast to the measurements reported by Ng (1978). The responses of the new Scots pine and lodegpole pine shoots were, however, not dissimilar from that found by Ng in his wind tunnel experiments, or to those found by Whiteman & Koller (1964) or Bennet & Rook (1978) with other Pinus species. The response of Sitka spruce was also very similar to that reported by Grace *et al* (1975) and by Watts & Neilson (1978) both of whom showed E reaching a plateau. Unfortunately no comparison can be found for the hybrid larch data. Thus the stomatal conductance results agree closely with other studies under controlled laboratory conditions, with the exception of Ng's work.

These stomatal responses are also similar to some of the field measurements, despite interactions in the field amongst other environmental variables. For example Rutter (1978) showed a similar diversity of response to D for the species he studied and the data of Watts *et al* (1976) for Sitka spruce is also very similar to the measurements for Sitka above, for the coincident range of D.

As there was no suggestion of a decline in E at large D there is no necessity to invoke a direct mechanism of stomatal response to D. The alternative hypothesis that the stomatal response to D might be mediated simply by a feedback system involving the bulk leaf water potential is, however, not adequate. Previous workers studying conifers (e.g. Beadle *et al*, 1981) found that the stomata were relatively insensitive to a change in water potential until the water potential fell below a critical level. This was about -1.6 MPa in Sitka spruce (Beadle *et al*, 1981) and at least -0.85 MPa for Scots pine (Ng, 1978). Therefore the small declines in leaf

water potential measured in this study are unlikely to be the cause of the stomatal closure found. An alternative hypothesis of response is developed later in this thesis.

The slope of the r_s/D relationship does not appear to be constant for these data. Therefore the hypothesis that one might be able to assume this to be constant for conifers (Roberts, 1983) is disproved. To check that this inconstancy is not unique to the data presented, the above data was extracted both from the publications Roberts referenced and also other papers. The results of linear regressions of r_s on D for these data are given in table 3.6b. It can be seen that both slope and intercept are highly variable when comparing the different studies. Some of this variation may be due to experimental technique; some may be due to errors in reading data from graphs in publications. However, the variation is so large that one can reject a theory of a constant slope for all species. The considerable variation between the values given by Roberts and those calculated from the same publications and shown here is unexplainable. Roberts also referenced Jarvis (1976) and Calder (1977) both of whom present models which are non-linear for r_s with respect to D. An attempt to extract a single representative value of dr_s/dD is given in table 3.6c. Clearly these values do not support his hypothesis either.

The analysis of r_s on D does, however, reveal that in most instances the linear regression provides a good description of the data when expressed in this way. With the exception of the models which are linear with respect to g_s, the coefficient of multiple determination (r^2) is larger for r_s on D than for g_s on D. Although this linearity is hard to interpret in terms of the physiology of the stomata, and is not directly related to E, the simplicity of a linear relationship may be useful for the development of predictive models. Schulze *et al* (1974) used such a model to predict daily trends in r_s.

Another use of the r_s/D curve is to determine if E declines as D increases. Farquhar (1978) showed that if a tangent to the curve crosses the x-axis for a value of D greater than zero then E will decline as D increases above the intercept value. The regression analysis gives some indication of whether this is likely to happen i.e. if the intercept of the

linear curve is negative then E will decline as D increases. For the regressions in table 3.6, none of the intercepts are significantly negative.

The decline in A observed as the stomata closed, also generally agrees with previous laboratory studies. A linear decline in A, not directly correlated to the non-linear decline in g_s, was also reported by Whiteman & Koller (1964), Watts & Neilson (1978), Bennett & Rook (1978) and Meizner (1982) (see above for species details). As shown in table 3.2 the reduction in A ranged from just over half of the reduction in g_s (in Sitka spruce) to approximately the same percentage (in hybrid larch). The limitation of A caused by stomatal closure can be analysed by studying the shape of the A/C_i relationship (see Chapter 9 and Jones, 1983).

More recently some workers have suggested another mechanism by which A can vary as D changes. Sharkey (1984) presented data that suggested that an increase in E may have a direct effect on A independent of stomatal closure. In addition one cannot rule out the possibility of an effect of feedback inhibition of photosynthesis by accumulation of photosynthates (see Neales & Incoll, 1968), or even photodestruction of pigments towards the end of the experiment. Such effects could have arisen because the shoots were subjected to a high photon flux density for a long period during measurement, compared to the growth room conditions. This complication interacting with the effect of D on g_s and A could, of course, have been avoided by randomising the order in which D was imposed on the shoot throughout the day. However, as the intention of the experiment was to follow the procedure followed by Ng, D was increased in even steps. The problem of the direction D is imposed and the diurnal complications are discussed in the next chapter.

Consequently analysis of the decline in A must be treated with some caution. However, the graphs of E/A against D (fig. 3.5) are similar to the curves which one can calculate for the data of Whiteman & Koller (1964), Watts & Neilson (1978), Bennet & Rook (1978) and Meizner (1982) and the general trend in all cases is for the ratio of E/A to increase, despite stomatal closure, as D increases.

The contrast between the old and new Scots pine data is interesting. The new Scots pine shoots have only approximately 60% of the photosynthetic rate of the older shoots. This is possibly the result of only partial development of the chlorophyll levels in the younger shoots, which were visibly less green. As a consequence of this, the ratio E/A is much larger and the slope as a function of D is also larger than for the older shoots, even though the younger shoots have a stronger response of g_s to D. The other species all have very similar E/A curves; only Sitka spruce shows a slightly lower slope, that is in part a reflection of the much stronger response of g_s to D.

The relevance of the shape of the E/A curve to a plant in the field is hard to analyse. It can be seen from fig. 3.5 that, in the short term, the stomata are unable to control E/A to a constant level as D is increased. The plant will therefore loose more water when D is large, per unit of CO_2 fixed. Over the time course of one day this may be of little importance as the plant may either be able to maintain a high enough supply of water to the leaves during the day to prevent a drop in water potential, or any decline caused by the increased evaporation may be recovered during the following night. However, in an arid environment such poor control may be critical to the plant's survival. Thus one might expect that the response of g_s to D might be a function of the conditions under which the plants have been grown. The comparatively weak responses to D, presented above might be a factor of the plants being well-watered compared to field grown trees. However, experiments done with radiata pine to test this hypothesis have been inconclusive (Rook, pers. comm.), though Hall *et al* (1975) and Schulze *et al* (1974, 1975a+b) reported increased sensitivity of stomata of *Citrus sinensis* L. and *Prunus armeniaca* L., respectively, to D, after pretreatments of high D.

To further analyse the long term effects of the response to D, other environmental variables must be considered. A mathematical approach to this problem is that outlined by Cowan (1977). The results of such an analysis for these data are presented in Chapter 10.

The analysis of stomatal sensitivity as a function of D shows (in fig. 3.6) that, with the exception of the old Scots pine, the stomata become less sensitive to D, as D increases. The sensitivity for the different plant materials is clearly different. The rate at which the sensitivity changes as D increases is also different and is roughly correlated with the absolute magnitude of sensitivity. Fig. 3.7 shows this to be the case. However, the relationship between dg_s/dD is clearly not the same for the different species, or even the different shoot ages of Scots pine. Thus the simple hypothesis of Morison & Gifford (1983) that dg_s/dD is a constant function of D does not hold when these data are also considered. As with the resistance regressions (see above), this is not really suprising as any comparison of g_s, r_s or related parameters depends on the determination of leaf area. Whether one uses total surface area or plan area the number of stomata considered in the estimate of conductance varies greatly, especially for interspecific comparisons, thus one expects considerable variation in absolute values of g_s. The dg_s/dD curves are, however, interesting when considered in relation to stomatal mechanisms and these graphs are discussed further, in relation to mechanisms, in Chapter 11.

To summarise, for the conifers studied a range of responses of g_s to D were found. For none of these did E decline as D increased i.e. there was no evidence implying a direct mechanism of stomatal response. However, no significant decline in bulk water potential occurred either, providing no evidence for feedback from a drop in bulk water potential. The decline in g_s, as D was increased was probably the main factor in causing a concurrent decrease in A. This results in E/A increasing as D increased.

The results were similar to many of the previous laboratory studies, with the notable exception of the work of Ng (1978). Thus experiments described in the following chapters were performed to investigate the differences between the responses he described and those above.

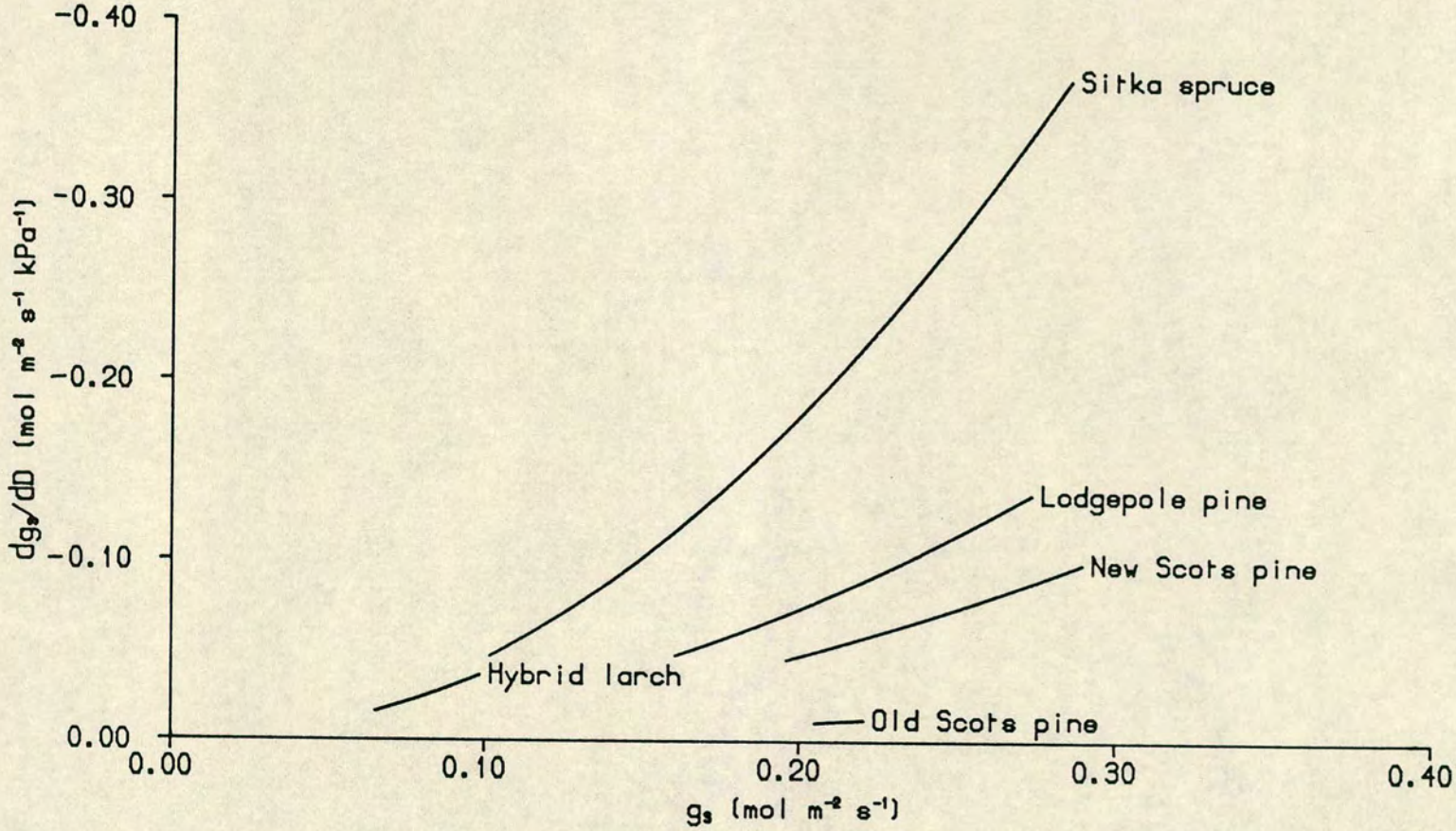

Figure 3.7: dg_s/dD as a function of g_s for a range of species. The curves represent transformations of the functions fitted to the E *versus* D data.

CHAPTER 4

HYSTERESIS IN THE RESPONSE OF STOMATA TO LEAF-TO-AIR VAPOUR PRESSURE DIFFERENCE?

4.1 Introduction

Although the results presented in Chapter 3 are broadly similar to many other laboratory experiments on conifers, there are still major discrepancies between these, and other laboratory experiments and many of the field studies. Much of these differences can be explained in terms of inadequate data analysis techniques and/or technical problems with field measurements (see Chapter 3). In addition there is another factor that must be considered when comparing field and laboratory experiments. In the field, plants are generally subject to continually varying D. Many of the data in papers presenting field-work report spot measurements of g_s, made at ambient D, using a porometer, i.e. not at a true steady-state. Laboratory measurements are, in the majority of cases, made under steady-state conditions with D being changed in predetermined steps, usually starting at low values of D which is increased in small but abrupt steps, during the time course of the experiment. The effects of this rather unnatural sequence of steps in D on the resultant response curve are rarely considered. Davies & Kozlowski (1974) briefly reported on differences in the dynamics of response of g_s to D for *Fraxinus americana* and *Acer saccharum*. They showed that not only was the rate of response different between the two species, but it varied with the direction in which the step in D was imposed. Sheriff (1977) however, reported that similar responses to D were measured for D being imposed in either direction.

To measure non-steady-state responses of stomata is technically very difficult, particularly when D is the independent variable because of the problems of water absorption and release from the measurement system. It is also very hard to define the treatment being imposed, because of its dynamic nature e.g. one must be able to control and specify the rate of change of D at the leaf surface. However, the first step in determining the response to dynamic changes in D, is to study the effect

of imposing changes, in D, in different directions.

As far as could be determined, none of the studies on conifers discuss the effects of the way in which D is imposed. However, in one study on a non-coniferous species, Schulze & Kuppers (1979), using *Corylus avellana* L., investigated the direction of changing D on the response. They showed that the response derived by increasing D was not always completely reversible. When D was decreased from high values, the resultant curve of E *versus* D did not always show a maximum value of E. Although variable their data do show that the measured response is dependent on the experimental procedure.

Two experiments were devised to test whether the direction of the imposed change in D had any effect on the response of the stomata of conifers to D. Firstly the response of g_s and A to D was measured, with D being changed in different directions, on seperate days for the same plant (this experiment will henceforth be called Expt. 1). Secondly, as a control, g_s and A were monitored over a period of 12 hours to see if, with constant D, there were any changes caused by factors such as feedback inhibition of photosynthesates, or gradual build up of localised water stress. This experiment was done at two different values of D for comparison (this will be refered to as Expt. 2).

4.2 **Plant material**

For comparison with Ng (1978) this work was done using (1+2)-year old-seedlings of Scots pine, provenance NT 10. The soil type and pretreatment growth conditions were as described in Chapter 3. For Expt. 1 the shoots used were 12-weeks-old, at the start of the experiment. The shoots broke bud outside and were transferred to the growth rooms 3 weeks prior to the experiment.

Expt. 2 was done at a different time of year. The shoots were 11-months-old at the start of the experiment. The differences in age of shoots must be borne in mind when comparing these experiments.

4.3 Experimental details

i) The direction of response.

The procedure adopted was broadly similar to that described in Chapter 3. Shoot preparation, pretreatment and watering regimes were identical. A shoot was placed in the chamber, in the dark, the previous evening and the lights switched on, on the morning of the experiment in the same way. The overnight value of D was set at 1.1 kPa for these experiments. Three hours after the lights were turned on measurements were made of g_s, E and A in the overnight conditions. D was then changed either to a higher value or a lower value depending on the replicate and treatment.

Four replicate shoots were used for this experiment. Experiments were done on sequential days on different shoots. For shoots one and two, D was initially increased from the overnight condition to *ca* 1.8 kPa, then decreased in four steps to 0.4 kPa. Four days later the reverse treatment was applied, i.e. D was initially increased to *ca* 0.4 kPa, then decreased in four steps to *ca* 1.8 kPa. For each step 100 minutes were allowed for equilibration. For shoots three and four the opposite sequence was applied, i.e. on the first day D was decreased in steps, then four days later D was increased in steps.

The xylem water potential was measured at the end of each experiment by taking three pairs of fasicles from the shoot being studied. This was done in order to check that the potential did not drop below the value considered to be a threshold for an effect on g_s (see Chapter 3).

ii) Daily trends in g_s and A.

The experiments were performed in an identical way to that above, except that after the lights were switched on, D was set to a predetermined value of either *ca* 1.0 kPa or *ca* 1.8 kPa and held at these conditions for 12 hours. These values were chosen as they were found to be relatively easy to hold constant during the timecourse of a day, yet would allow a reasonable comparison of the effect of D. gs, E and A were

monitored throughout the whole of this period. However, because of excessive zero drift of the IRGA during these experiments, data are only presented for averages for 20 minute periods taken every 3 hours, shortly after the IRGA had been calibrated.

During the intervening period between the two experiments it became apparent that changes in the concentration of CO_2 in the ambient air could mask slight changes in A, caused by effects other than changing CO_2 concentration. Although ambient CO_2 was found to vary by only ±20 µmol mol^{-1} under normal circumstances, to rule out this possiblity the air supply to the gas exchange system was changed to air generated by the gas mixing pumps. A fixed concentration of 350 µmol mol^{-1} was used for all the experiments.

Three replicates were used for this experiment. For two of the replicates the daily trends were measured intially at 1.0 kPa, then three days later at 1.8 kPa. For the other replicate D was set to 1.8 kPa and then to 1.0 kPa three days later. This was done to check if there was an effect of subjecting the plant to a large value of D that could be detected three days later.

Needle xylem water potentials were also measured at the end of each day by taking three fasicles from the shoot that had been studied.

4.4 Results

i) Experiment 1: the direction of response.

Graphs of g_s, E and A as a function of D are presented for experiments performed in different directions in figures 4.1, 4.2 and 4.3 respectively. A graph of E/A is also presented in figure 4.4. These graphs have been generated by standardising the data to the starting value at 1.1 kPa, using the procedure outlined in Chapter 2. The actual mean values (with standard errors) for the replicates at the starting condition were for g_s 0.315 (±0.022) mol m^{-2} s^{-1} and for A 19.66 (±1.17) µmol m^{-2} s^{-1}.

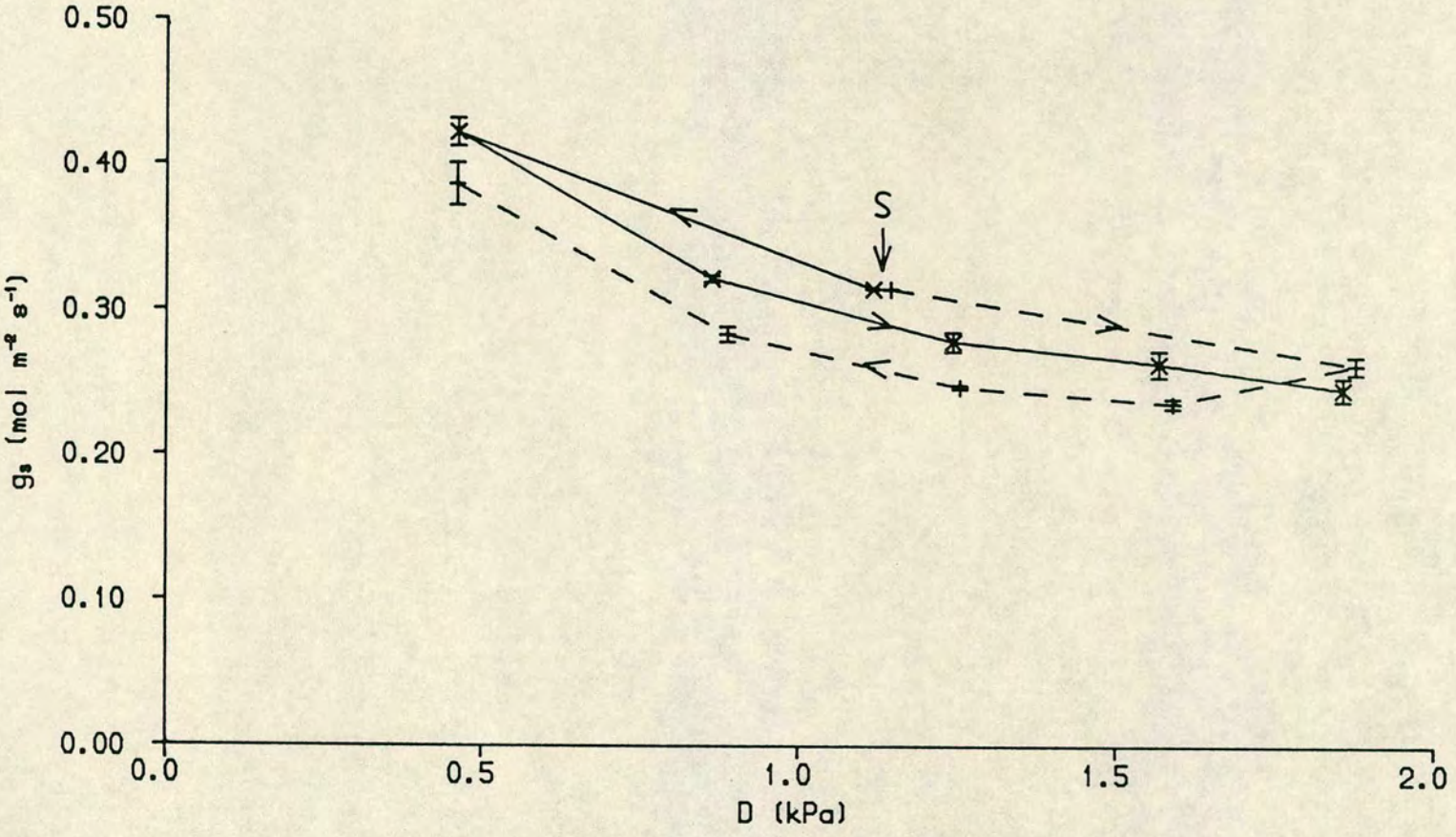

Figure 4.1: g_s as a function of D for two experiments where D was imposed in different directions (indicated by the arrow-heads and line-type). 'S' indicates the level of D at the start of the experiment. The data points represent the mean of 3 replicates, plus 1 S.E. The points are joined by straight lines.

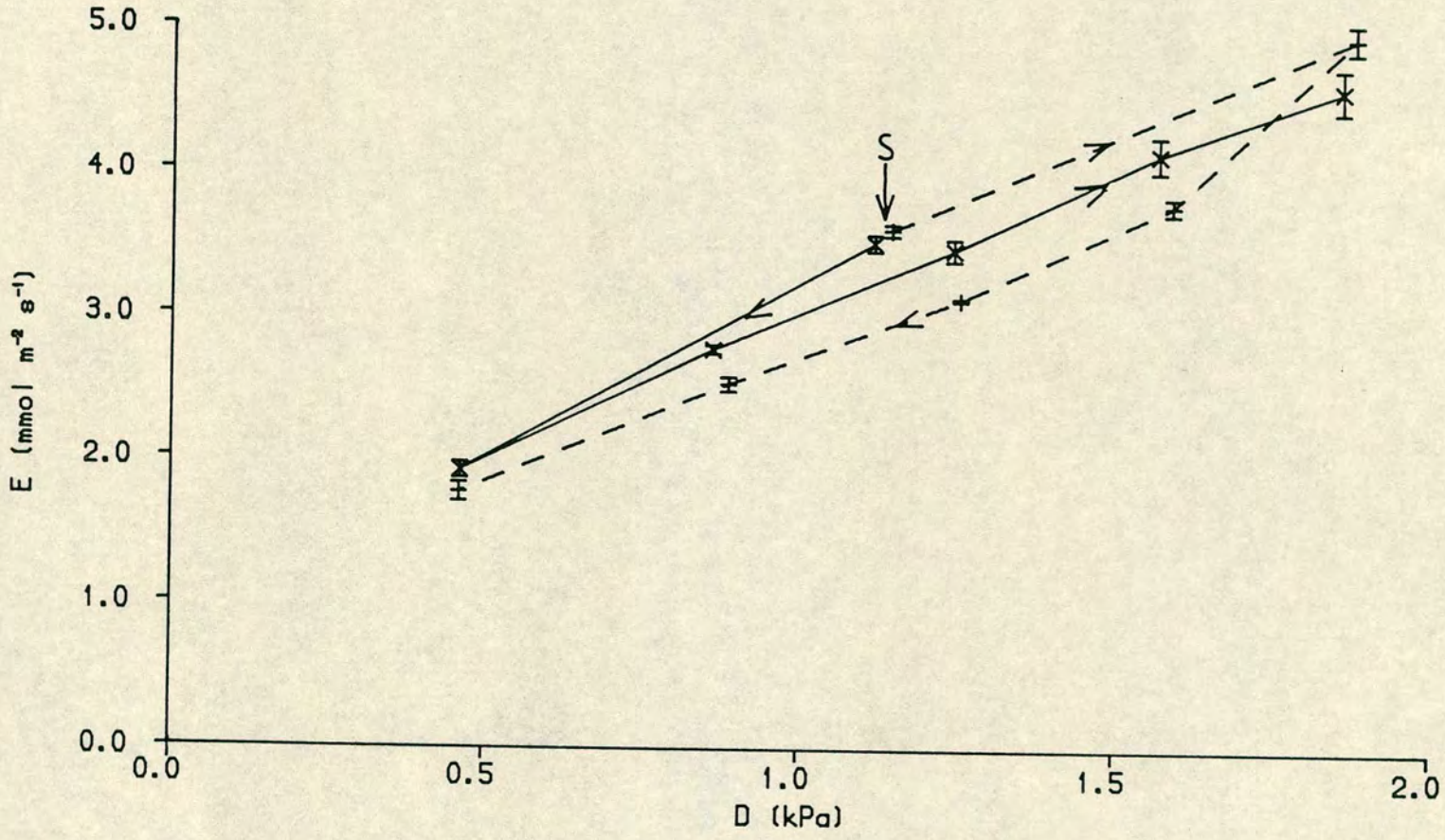

Figure 4.2: E as a function of D for two experiments where D was imposed in different directions (indicated by the arrow-heads and line-type). 'S' indicates the level of D at the start of the experiment. The data points represent the mean of 3 replicates, plus 1 S.E. The points are joined by straight lines.

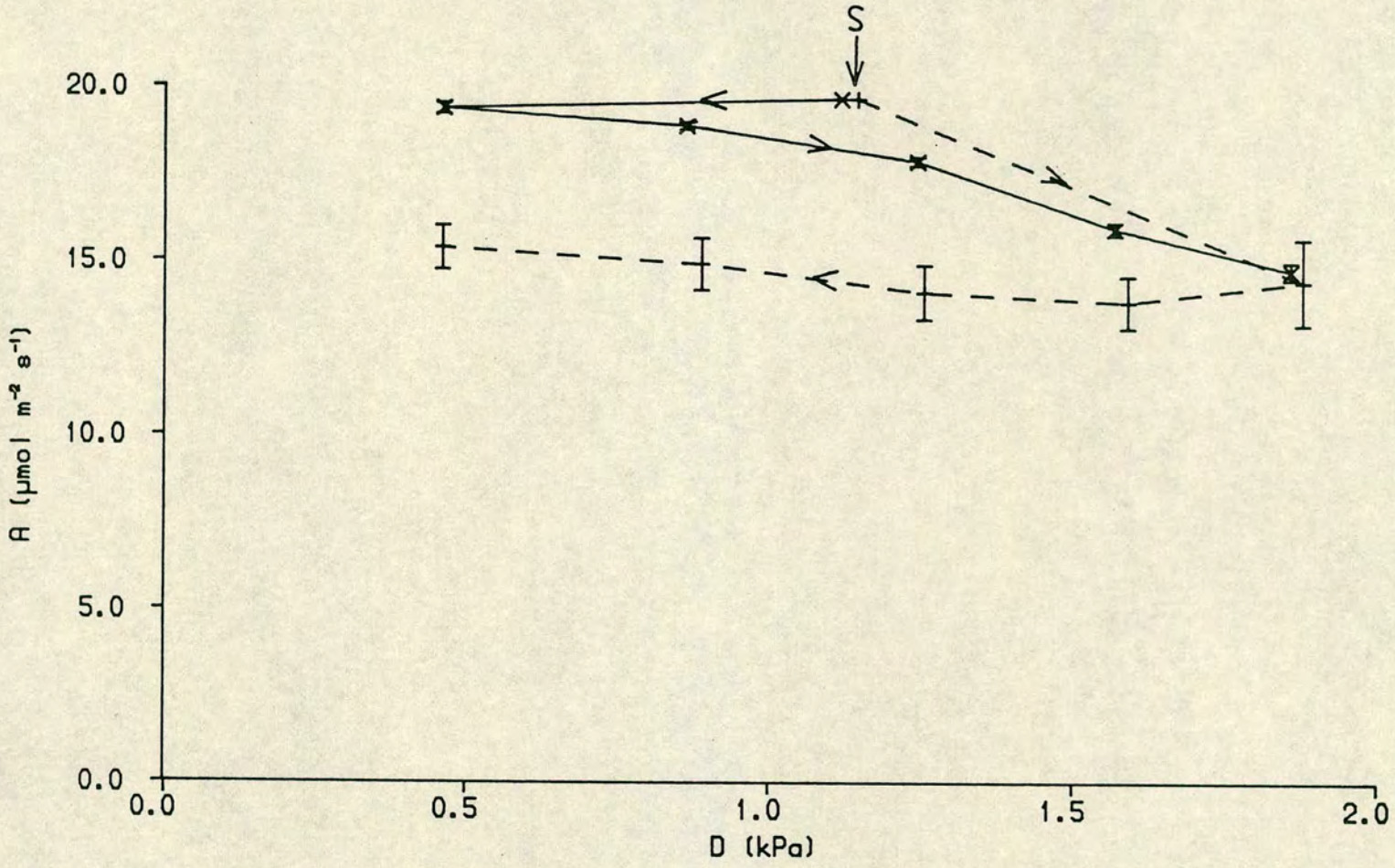

Figure 4.3: A as a function of D for two experiments where D was imposed in different directions (indicated by the arrow-heads and line-type). 'S' indicates the level of D at the start of the experiment. The data points represent the mean of 3 replicates, plus 1 S.E. The points are joined by straight lines.

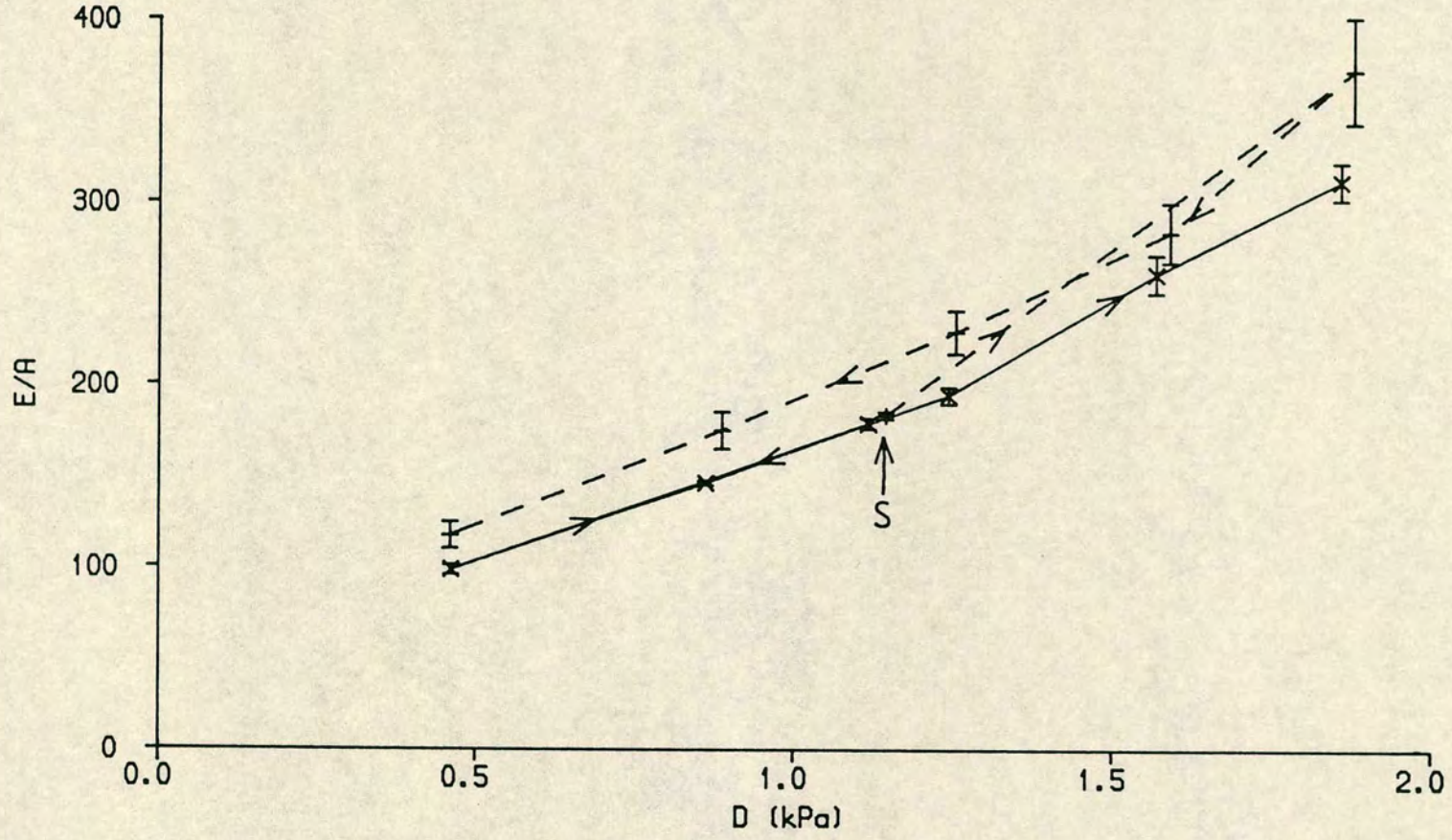

Figure 4.4: E/A as a function of D for two experiments where D was imposed in different directions (indicated by the arrow-heads and line-type). 'S' indicates the level of D at the start of the experiment. The data points represent the mean of 3 replicates, plus 1 S.E. The points are joined by straight lines.

The graph of g_s as a function of D (fig. 4.1) shows that for increasing D the response was broadly similar to the response shown in Chapter 3, for the slightly younger Scots pine shoots, i.e. there was stomatal closure of *ca* 40% as D was increased from 0.4 to 1.8 kPa. The graph gives an indication that g_s is generally lower when D is decreased. This is also reflected in the graph for E (fig. 4.2).

The curves for the response of A to D (fig. 4.3) show differences between the direction in which D was changed. Again when D was increased from 0.4 to 1.8 kPa the response was broadly similar to those shown in Chapter 3, i.e. showing a linear decline in A of *ca* 25% over that range. However when D was changed in the reverse direction, A first declined by *ca* 27% when D was increased from the starting value of 1.1 to 1.8 kPa and then remained at a more or less constant value as D was decreased although the stomata eventually opened to a larger conductance than at the start.

The graph of E/A (fig. 4.4) shows this clearly as after the initial jump from 1.1 to 1.8 kPa, E/A remained consistently higher as D is decreased in comparison with the repsonse measured in the reverse direction.

Bearing in mind the limitations of applying 'normal' statistics to standardised data, as discussed in Chapter 2, an analysis of variance was applied to the data. A multiway analysis of variance was applied with the sequence of treatments of D, i.e. on day one or on day four, the direction in which D was applied and D were all considered as factors in the experimental design. The analysis was applied to the standardised data, but with the initial measurement, at 1.1 kPa, ignored as this value is the same for all replicates and treatments. The results of the analysis are presented in table 4.1.

The analysis shows that the sequence in which the experiments were done had no significant effect on either g_s or A. For g_s there was a significant effect due to D, although this was not matched for A. For both g_s and A there was, however, a significant effect of the direction in which D was applied: g_s and A were significantly smaller when D was decreased from a high to low value. The interaction between D and

Table 4.1 The results of an analysis of variance to test for the effects of the direction in which D was imposed on the response of g_s and A to D. The table is extracted from the output of the Genstat statistical package used for the analysis (see Appendix 4).

VARIATE: g_s

SOURCE OF VARIATION	DEGREES OF FREEDOM	VARIANCE RATIO	LEVEL OF SIGNIFICANCE p <
EXPERIMENTAL FACTORS			
SEQUENCE	1	0.288	N.S.
DIRECTION	1	5.679	0.025
D	4	35.260	0.001
DIRECTION.D	4	1.096	N.S.
RESIDUAL	29		
TOTAL	39		
GRAND TOTAL	39		
GRAND MEAN		0.2954	
TOT. NO. OF OBSERVATIONS		40	

VARIATE: A

SOURCE OF VARIATION	DEGREES OF FREEDOM	VARIANCE RATIO	LEVEL OF SIGNIFICANCE p <
EXPERIMENTAL FACTORS			
SEQUENCE	1	0.544	N.S.
DIRECTION	1	12.981	0.005
D	4	1.951	N.S.
DIRECTION.D	4	0.841	N.S.
RESIDUAL	29		
TOTAL	39		
GRAND TOTAL	39		
GRAND MEAN		15.94	
TOTAL NUMBER OF OBSERVATIONS		40	

N.S. - Not significant at $p < 0.10$

direction was not significant though, for either g_s or A, showing that there was no simple interaction between D and direction of treatment, i.e. the degree of response to D was not larger depending on direction.

The lowest water potential measured for any of the fascicles taken from the shoots at the end of each days' measurements was -0.72 MPa, i.e. the water potential did not drop below the so-called threshold value discussed in Chapter 3.

ii) Experiment 2: daily trends

The daily trends for g_s and A are shown in figures 4.5 and 4.6 respectively, for each shoot. These graphs also show the considerable shoot-to-shoot variation encountered in all experiments reported in this thesis. The mean values for each time are given in table 4.2a. The mean percentage declines for both g_s and A, from 180 min after the lights were turned on, to 720 min after the lights were turned on are given in table 4.2b.

Table 4.2a The mean values of g_s and A for the three replicates at given times after the lights were turned on. The data used were unstandardized; standard errors are given in brackets.

Time (min)	g_s (mmol m^{-2} s^{-1})		A (µmol m^{-2} s^{-1})	
	1.0 kPa	1.8 kPa	1.0 kPa	1.8 kPa
180	115.8 (±15.4)	100.2 (±20.0)	7.32 (±.93)	7.57 (±.40)
360	115.1 (±16.5)	85.5 (±17.8)	7.40 (±.94)	5.96 (±.40)
540	114.5 (±14.4)	79.6 (±15.7)	7.34 (±.82)	6.61 (±.36)
720	111.6 (±13.1)	75.6 (±14.2)	7.59 (±.76)	6.71 (±.54)

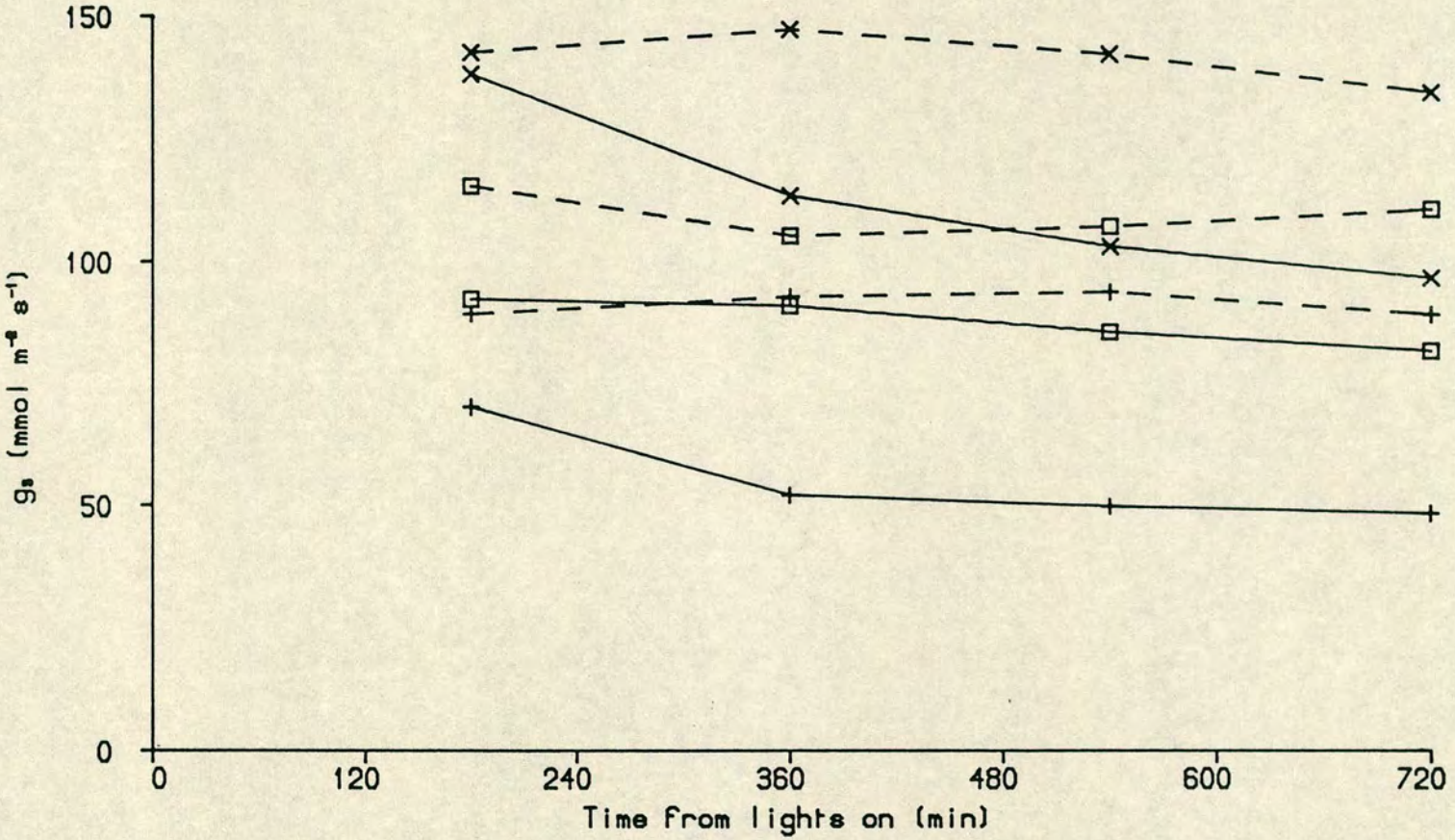

Figure 4.5: The time course of g_s for three shoots, at two different levels of D. For the solid line D = 1.8 kPA, for the dashed line D = 1.0 kPa.

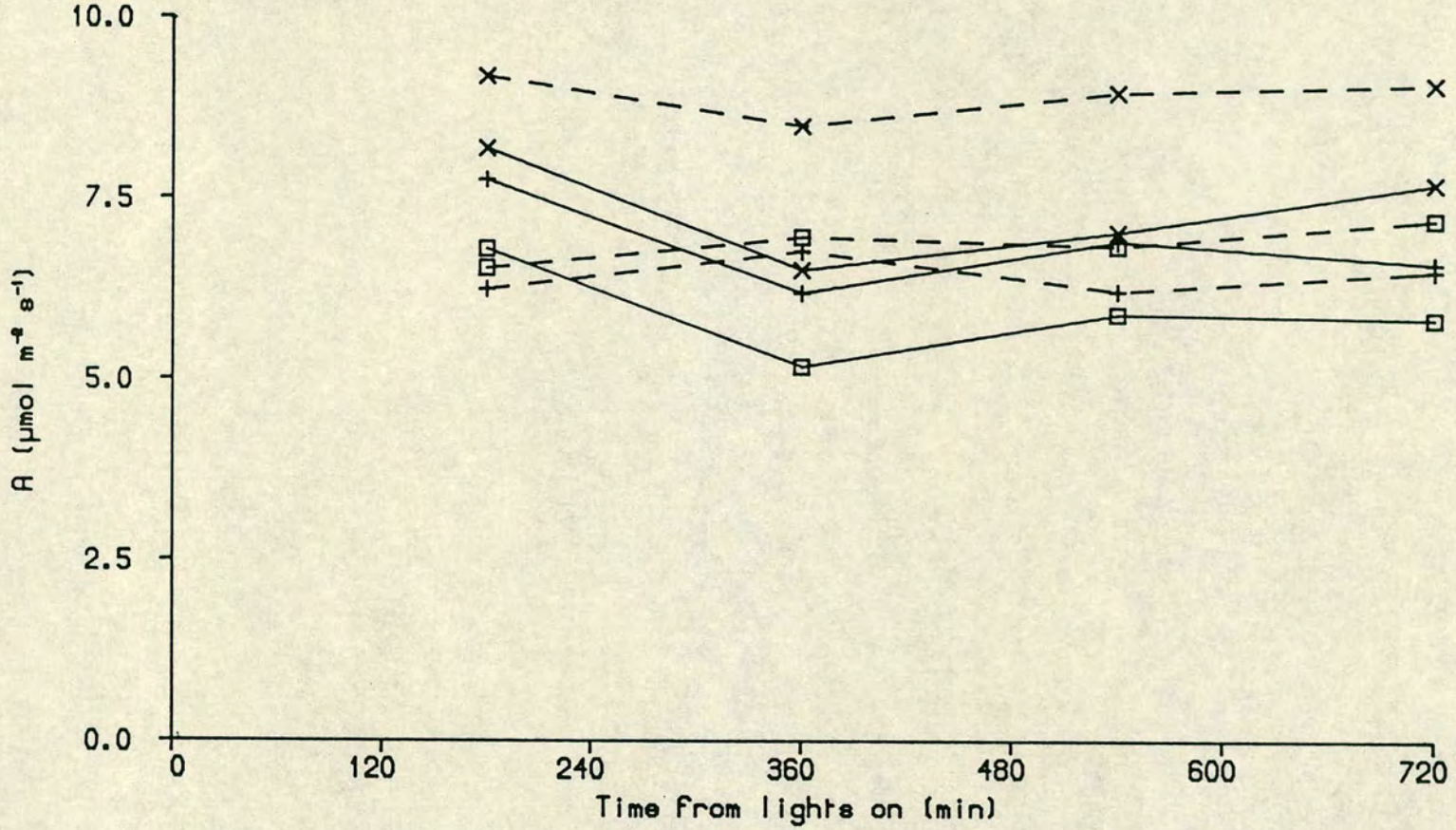

Figure 4.6: The time course of A for three shoots, at two different levels of D. For the solid line D = 1.8 kPA, for the dashed line D = 1.0 kPa.

Table 4.2b The percentage change in the measured values over the period from 180 to 720 min after the lights were turned on. The values used were the means given in table 4.2a.

	D (kPa)	
	1.0	1.8
% change in g_s	-3.6	-24.6
% change in A	+3.7	-11.4

It is interesting to note that the decline in g_s represents a much larger decline in response to the increase in D than was reported in Chapter 3 for Scots pine shoots of this age.

To see if there was any significant effect of time on g_s and A, an analysis of variance was applied to these data. To avoid the problem of the considerable shoot-to-shoot variation the replicates were treated as 'blocks' rather than as replicates of the same block (see Genstat manual, ref. Appendix 4). The results of the analysis are shown in table 4.3.

As expected D has a significant effect on both g_s and A. Time, however, only has a significant effect on g_s, with no interaction with D, i.e. the reduction in g_s is just as significant for both levels of D. For A neither time nor any interaction with D was significant.

Although not presented in table 4.3, the possiblity that the sequence in which the experiments were done (low or high D on day one) was investigated. As in Expt. 1 (above) this was not found to be significant so this factor was removed from the analysis.

The lowest water potential measured for all shoots after experiments at low or high D was -0.68 MPa, again above the threshold discussed in Chapter 3.

4.5 Discussion

The results for Expt. 1 show a small, but none-the-less significant difference in response for both g_s and A to D, depending on the

Table 4.3a The results of the analysis of variance to test if g_s changes over the course of a day. The table is extracted from the output of a run of the Genstat statistical package (see Appendix 4).

VARIATE: g_s

SOURCE OF VARIATION	DEGREES OF FREEDOM	VARIANCE RATIO	LEVEL OF SIGNIFICANCE p <
REPLICATE STRATUM	2	125.551	0.001
EXPERIMENTAL FACTORS			
D	1	108.416	0.001
TIME	3	4.861	0.025
D.TIME	3	2.813	0.10
RESIDUAL	14		
TOTAL	21		
GRAND TOTAL	23		
GRAND MEAN		99.8	
TOTAL NUMBER OF OBSERVATIONS		24	

Table 4.3b The results of the analysis of variance to test if A changes over the course of a day. The table is extracted from the output of a run of the Genstat statistical package (see Appendix 4).

VARIATE: A

SOURCE OF VARIATION	DEGREES OF FREEDOM	VARIANCE RATIO	LEVEL OF SIGNIFICANCE p <
REPLICATE STRATUM	2	19.521	0.001
EXPERIMENTAL FACTORS			
D	1	8.138	0.025
TIME	3	1.699	N.S.
D.TIME	3	2.083	N.S.
RESIDUAL	14		
TOTAL	21		
GRAND TOTAL	23		
GRAND MEAN		7.07	
TOTAL NUMBER OF OBSERVATIONS		24	

N.S. - not significant at p < 0.10

direction in which the treatments were imposed.

The different responses could possibly be explained by a carry-over effect, following the imposition of the large increase in D at the start of the experiments in which D was decreased in steps. Evidence for such an effect can be seen in the shape of the g_s graph. The stomata did not open, and may, possibly, even have continued to close after D was initially increased to 1.8 kPa and then decreased to 1.4 kPa. The initial large step in D might have led to an increase in E sufficient to cause some localised water stress within the leaf, before the stomata had time to respond to the change in D. The carry-over effect could be caused by the stomata over-responding to such a stress.

A similar response to a large step change in the environment was also often seen in many other experiments when the lights were switched on from total darkness. The stomata were seen to open fairly rapidly, overshoot and then close to an intermediate conductance over the timecourse of one to two hours. Expt. 2 also showed a trend for the stomata to open too far in the morning (see fig. 4.5). This trend is more apparent for the high D treatment in which the plants were subjected to a large step in both light and D. In control theory terms this is an example of an overshoot caused by a sharp step in the prevailing conditions within the system being controlled (Cowan, 1972).

The large step from 1.1 to 1.8 kPa also appears to have had a carry-over effect on A. A did not subsequently increase, even though the stomata opened further in response to a decrease in a to *ca* 0.8 kPa. The fact that A did not recover, despite the increase in g_s, implies that there had been some damage to the photosynthetic apparatus that was not reversible in the short term. This damage might, possibly, have been caused by stress resulting from an uncontrolled burst in E, as proposed above.

Thus the difference in response between the two directions of measurement may have been a result of the imposition of a large stepped increase in D, rather than the direction in which D was applied. If this is the case then great care must be taken in designing and running

experiments where D is a variable. In particular the idea of imposing treatments of D randomly, rather than in an ordered sequence, may well cause more problems than it solves, as this will inevitably involve changing D in some very big steps.

It is possible that some of the responses to D reported in the literature may be influenced by this effect. For example, Sharkey (1984) showed a decrease in A, for the same C_i, when E was increased. This might be the result of a large rapid change in D leading to local desiccation.

Preliminary experiments were done with broad-leaved species using a null-balance porometer. In such a system D could be increased rapidly. Large increases in D were often found to cause stomata to oscillate wildy. Large decreases in D did not cause such oscillations. This can be interpreted as showing that even species with comparatively fast responding stomata (cf. conifers) may be unable to maintain steady control of water loss under such conditions, with possible damage resulting to the photosynthetic apparatus.

This suggests caution in the use of ventilated porometers. In the porometer chamber, the windspeed may be somewhat increased above the ambient conditions. (The quoted windspeed for the flat-leaf chambers of the Li-Cor LI-1600 null-balance porometer is 6 m s^{-1}). This may break down the natural boundary layer around the leaf, thereby increasing D at the leaf surface and causing a rapid increase in E. This problem may be another factor in explaining some of the differences between the laboratory and field experiments discussed in Chapter 3.

For conifers the rapid changes in D imposed on the shoot in these experiments are not a common occurrence in the field. The canopy of a conifer stand is relatively well-coupled to the atmosphere above. Even with rapid changes of weather, such as a front going through, D is unlikely to change in large steps in a matter of tens of seconds. Therefore the possible damage outlined above, is not likely to be of importance to the functioning of a leaf and this may be one reason why the stomata are so slow to respond. In contrast, species growing in short

vegetation often create their own local microclimate. This microclimate can be broken down very rapidly, i.e. by a gust of wind, so that the ability to cope with rapid changes in D may be more important and probably deserves more investigation.

It seems that the direction in which D changes is only significant in that large increases in D may by detrimental, whilst large decreases are likely to be harmless. Experimentally the direction in which D is applied seems of little importance as long as D is not increased in large steps. For a Scots pine tree in the field it also seems that the direction in which D changes is of small importance, although this may not hold for other species, especially those with stronger responses to D.

Expt. 2 shows that there is also the possible complication of a slight decline in g_s over the course of a day. It also seems that the decline is larger with high D - this is evident from the graph and the analysis of variance, though only significant at the 10% level. The level of decline in g_s at low D is rather small (see table 4.2b) and can be considered insignificant. At high D the decline is big enough to affect measurements seriously .

One explanation is that there is a diurnal rhythm of stomatal action that is promoting closure towards the end of the day, although this would not explain the larger decline of g_s at high D, unless one introduces the possiblity of sensitivity to D being linked to the time of day. An alternative explanation for this decline is that the high value of D imposed is outside the range normally experienced by these plants. Thus stomatal control is not adequate to prevent a gradual build up in stress over the timecourse of a day. This stress can only be local as the measurements of bulk water potential at the end of the day did not show a level of stress likely to cause stomatal closure.

The decline in A was not significant, although the mean values were lower at the end of the day. As a large part of this decline may be accounted for by the stomatal closure, the possibility of photodestruction, or feedback inhibition of photosynthesates can be considered as insignificant in this experiment.

The strong apparent response to D of these shoots in comparison with that reported for 11-month-old shoots in Chapter 3 could be partly the results of the large increase in D at the start of the day, as in Expt. 1.

These two experiments show that one must be careful in designing an experiment. One must try not to impose unnaturally large, rapid steps in D and also avoid subjecting plants grown under low D to high D for long periods. Both these conditions were met for the experiments described in Chapter 3, and for all other experiments presented in this thesis.

CHAPTER 5

INTERACTIONS BETWEEN THE RESPONSE TO PHOTON FLUX DENSITY AND LEAF-TO-AIR VAPOUR PRESSURE DIFFERENCE

5.1 Introduction

An important environmental variable which is also often not considered when isolating responses of the stomata to D, from field data, is photon flux density (Q). This is suprising as Q was one of the first variables to which stomata were found to respond and has since been much studied (a recent summary of the possible mechanisms of the response to Q can be found in Willmer (1983)). Not only is there a possibilty that field data where Q was variable, may have extra scatter in the data as a result of g_s being a function of Q, but there is also a possibility that the response to D may interact with Q.

Evidence, in the literature, for an interaction between Q and D is fairly limited and somewhat conflicting. One of the problems in analysing the data presented, is defining what is an interaction between the responses to Q and D. If Q is low and g_s small, it may be physically impossible for the stomata to close, in absolute terms, to the same degree as if they were fully open at high Q. Thus for my purposes an interaction is defined, not in the additive way as by the traditional statistical definition, but in functional terms as a greater or lesser percentage change of g_s (or E or A) as D is changed from one level to another, at different levels of Q. This definition is used as there is evidence to suggest that the sensitivity of stomata to D, is proportional to the absolute value of conductance (see Chapter 3 and also Morison & Gifford, 1983).

On this basis Kaufmann (1976) showed a much stronger response of g_s to D for shoots of Engelmann spruce in 'the shade' than 'in sunlight' in the field (his fig. 1). However he did not make clear whether Q was manipulated to create such conditions, or whether these terms refered to different kinds of foliage. In contrast, data were also presented in the same paper (his fig. 4), for growth chamber measurements, which showed

no statistical interaction between Q and D on g_s. (There may have been technical problems with these measurements - see Chapter 3.).

For Scots pine Ng (1978) showed that the response of g_s to D was greater at high Q, rather than at low Q, to the extent that the stomata did not appear to respond to D at all when Q was as low as 110 $\mu mol\ m^{-2}\ s^{-1}$. Some caution must be applied to the interpretation of these data though, as the data was extracted from experiments primarily designed to measure the light response curves of the stomata, i.e. D was held constant on one day while Q was varied. However Rutter's (1982) field data for incense-cedar, ponderosa pine and white fir do not appear to show any difference in response to D at different levels of Q.

Reports of such studies for non-coniferous species are also rare. Davies & Kozlowski (1974) state that both *Fraxinus americana* and *Acer saccharum* were 'more sensitive' to D at low rather than high Q, though no data were presented to support this statement.

Thus not only has the possibilty of an interaction not been widely investigated but the results that do exist are somewhat conflicting. As such an interaction is not only important to the control of water loss and CO_2 fixation by a plant in the field, but also to our ability to be able to describe the plant's behaviour using modelling techniques (Jarvis, 1976), it was considered to be of value to investigate the possible interaction further. Also the possibility that there might be some major physiological difference between the plants used in these studies and those used by Ng (1978) was being aired at this stage of the project. As some of the literature suggested that g_s might be more sensitive to D at low Q, I decided to see if the strong responses of g_s to D described by Ng could be reproduced at lower Q.

5.2 **Plant material**

For comparison with the work presented in Chapters 3 and 4, (1+2)-year-old Scots pine seedlings of provenance NT10 were used. The shoots used were 10.5 months old at the start of the experiments. They

had broken bud naturally outside the previous year. The soil and pretreatment conditions were identical to those described in Chapter 3.

5.3 Experimental details

One shoot was selected from each of four plants. The response curves of g_s, E and A to D were measured in an identical way to that described in Chapter 3. The shoot preparation and experimental procedures were all the same. For all experiments D was initially decreased from the overnight value of 1.0 kPa, then increased in 5 steps to approximately 2.0 kPa.

For each shoot the responses to D were measured at four different levels of Q; Q being held constant for each days measurements. The interval between measurement for each shoot was four days. The order in which the different levels of Q were applied was chosen randomly.

The different levels of Q were achieved by placing neutral density filters between the light source and the shoot chamber. The filters gave approximately 55, 28 and 9 % of the unfiltered Q. These steps were chosen for comparison with Ng (1978). The mean values of Q are given in table 5.1.

The xylem water potentials of three fascicles taken from the shoot were measured at the end of each experiment.

5.4 Results

The responses of g_s, E, A and E/A for the four levels of Q are shown in figures 5.1, 5.2, 5.3 and 5.4, respectively. These graphs represent the means of data which have been standardised in the way described in Chapter 2. The reference condition for standardisation was the lowest value of D and highest level of Q.

Curves were fitted to the data in the following ways:

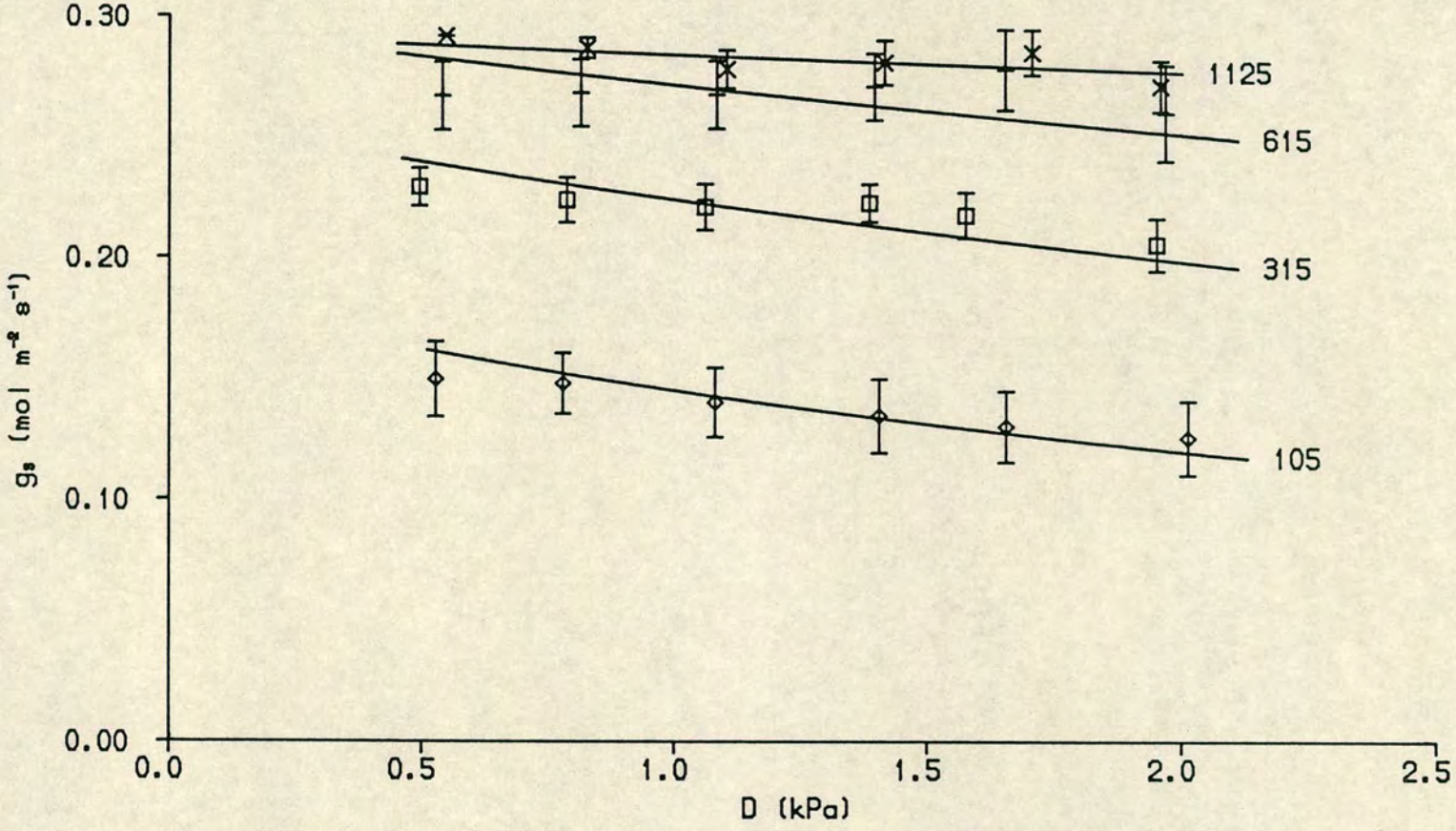

Figure 5.1: g_s as a function of D for four levels of Q (μmol m^{-2} s^{-1}). Data points represent the mean of 4 replicates, plus 1 S.E. See the text for a description of the fitted curves.

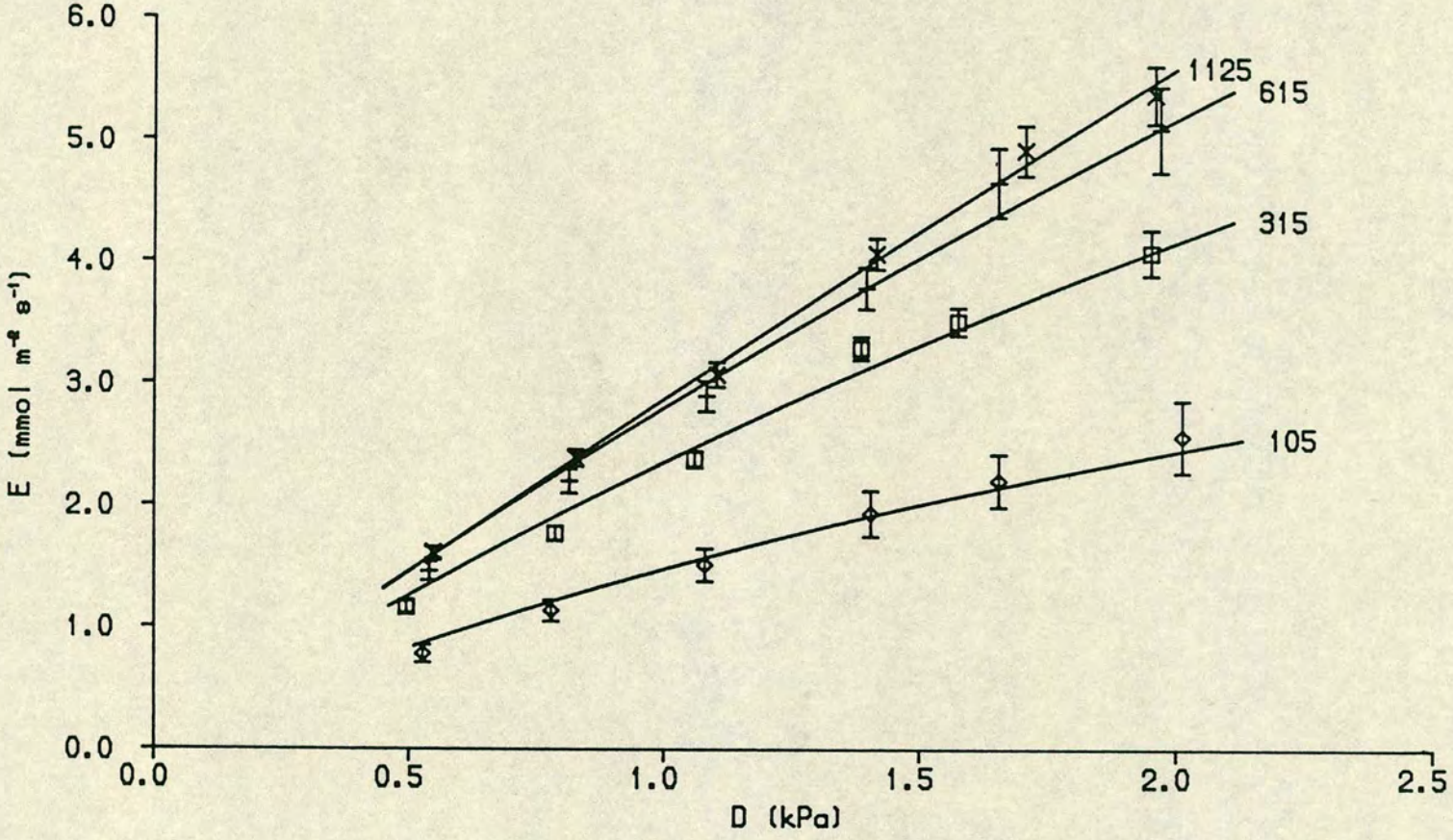

Figure 5.2: E as a function of D for four levels of Q (μmol m⁻² s⁻¹). Data points represent the mean of 4 replicates, plus 1 S.E. See the text for a description of the fitted curves.

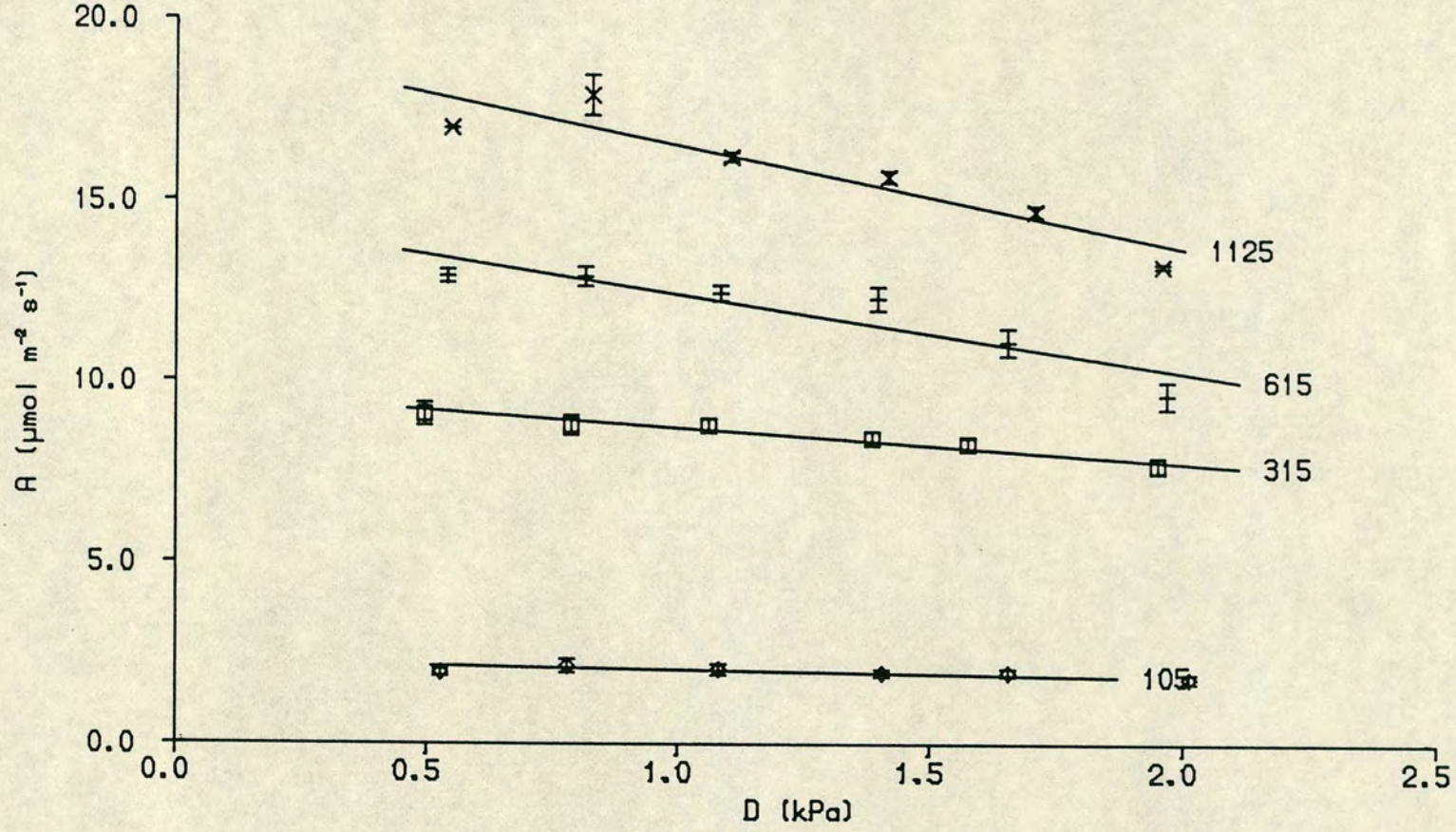

Figure 5.3: A as a function of D for four levels of Q (umol m⁻² s⁻¹). Data points represent the mean of 4 replicates, plus 1 S.E. See the text for a description of the fitted curves.

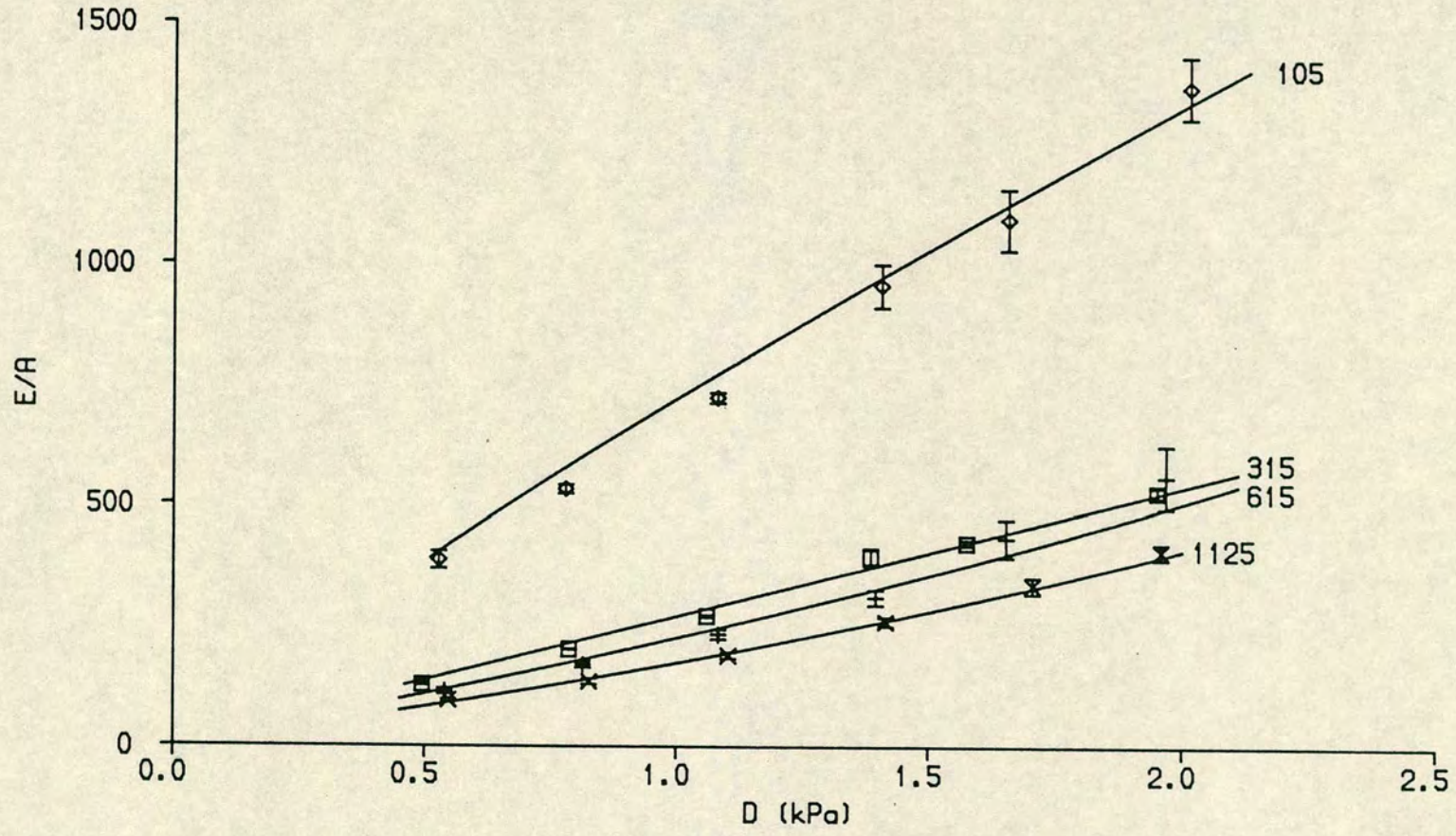

Figure 5.4: E/A as a function of D for four levels of Q (μmol m^{-2} s^{-1}). Data points represent the mean of 4 replicates, plus 1 S.E. See the text for a description of the fitted curves.

In the case of g_s and E, the same technique was used as described in Chapter 3, i.e. by fitting a rectangular hyperbola to the E/D data (equation 3.1). The g_s/D curves were generated from the E/D curves as using equation 3.2. The rectangular hyperbola was found to give the smallest mean square error for all replicates compared to linear and non-rectangular hyperbolic curves. The difference in the mean square errors between the fits was small in some cases, but it was decided to use the same equation to allow direct comparison to be made amongst all the data in this thesis. The parameters for the fitted curves are given in table 5.1.

Table 5.1 The parameters derived from fitting hyperbolic curves, of the form of equation 3.1, to the E *versus* D data for each level of Q. The asymtopic standard deviations of the parameters are given in the brackets. For Q the mean value for the four replicates are given with ± one standard error in brackets. Units for Q are μmol m^{-2} s^{-1}, for E_m are mmol m^{-2} s^{-1} and for a are mmol m^{-2} s^{-1} kPa^{-1}. N=24.

Q	E_m	a
1124 (±32)	107.9 (±277.3)	2.951 (±0.337)
614 (±18)	34.3 (±50.1)	3.049 (±0.625)
315 (±7)	18.1 (±11.6)	2.706 (±0.395)
104 (±4)	7.1 (±6.5)	1.873 (±0.703)

For A/D data linear curves were fitted, again allowing direct comparison with Chapter 3. The parameters for these curves are given in table 5.2.

Table 5.2 The parameters derived from fitting a linear regression of A as a function of D, for different levels of Q. ±one standard error is given in brackets. The units and standard errors for Q are as in table 5.1. The units for the slope are μmol m^{-2} s^{-1} kPa^{-1}. The units for the intercept are as for A, i.e. μmol m^{-2} s^{-1}.

Q	Slope	Intercept	r^2
1124	-2.821(±0.459)	19.35(±0.62)	0.6316
614	-2.165(±0.504)	14.57(±0.67)	0.4563
315	-0.959(±0.345)	9.66(±0.45)	0.2602
104	-0.211(±0.191)	2.26(±0.26)	0.0524

The curves for the E/A *versus* D data were calculated from the curves fitted to E and to A *versus* D.

An attempt had to be made to analyse the data further, by applying an analysis of variance to both the standardised g_s and A data. As stated in Chapter 2, statistical analysis of standardised data of this form, collected from sequential measurements is not truly valid. The results of the analysis of variance are given in table 5.3. They show that there was no significant response of g_s to D and therefore no significant interaction between D and Q. There was however a significant response of A to D and also a significant interaction between D and Q in their effects on A.

The lowest xylem water potential measured at the end of the days' measurements, for all of the experiments, was -0.79 MPa. This is above the threshold level for stomatal closure that is described by Ng (1978).

5.5 Discussion

The response of the stomata to D (fig. 5.1) at high Q, was very similar to that reported in Chapter 3 for Scots pine shoots of this age, i.e. there was no significant response. The stomata appear to be almost light-saturated at 614 μmol m^{-2} s^{-1} justifying the assumption, made in other experiments in this thesis, that at 1124 μmol m^{-2} s^{-1} light is not likely to be limiting stomatal aperture. However, A does not appear to be totally saturated at 614 μmol m^{-2} s^{-1} as A is *ca* 25% higher at maximum

Table 5.3 The results of an analysis of variance to test for the effects of the photon flux density on the response of g_s and A to D. The table is extracted from the output of the Genstat statistical package used for the analysis (see Appendix 4).

VARIATE: g_s

SOURCE OF VARIATION	DEGREES OF FREEDOM	VARIANCE RATIO	LEVEL OF SIGNIFICANCE p <
EXPERIMENTAL FACTORS			
Photon flux	3	42.524	0.001
D	5	0.273	N.S.
Photon flux.D	15	0.043	N.S.
RESIDUAL	72		
TOTAL	95		
GRAND MEAN		0.2276	
TOTAL NUMBER OF OBSERVATIONS		96	

VARIATE: A

SOURCE OF VARIATION	DEGREES OF FREEDOM	VARIANCE RATIO	LEVEL OF SIGNIFICANCE p <
EXPERIMENTAL FACTORS			
Photon flux	3	910.856	0.001
D	5	13.356	0.001
Photon flux.D	15	2.459	0.01
RESIDUAL	72		
TOTAL	95		
GRAND MEAN		9.546	
TOTAL NUMBER OF OBSERVATIONS		96	

N.S. - Not significant at p < 0.10

Q. The reason for this is uncertain as Q was only 300 μmol m^{-2} s^{-1} in the preconditioning growth room and it was hoped that the plants would have acclimatised to these conditions in three weeks, i.e. A would be saturated at a much lower level. However, it is not uncommon for g_s to saturate at lower Q than A, so the relative levels at which g_s and A become light saturated is not abnormal. In addition interactions between g_s and A, via C_i, are likely to be minimal because of the small degree of feedback between g_s and C_i in Scots pine (Ng, 1978).

The graphs of E as a function of D (fig. 5.2) show slight curvature at the lowest values of Q. The fitted graphs of g_s *versus* D (fig. 5.1) also reflect this trend, but in addition show some problems in the fit of the curves to the data, as there are clear discrepancies between the data points and the lines. In fact straight lines, virtually parallel to the x-axis would visually appear to fit better. The poor fit is probably a result of the regression analysis being less-sensitive to the values of E at low D, where E is comparatively small. When the fitted curve are transformed to g_s *versus* D these errors, at low D, are amplified. As E is low under these conditions anyway, this is likely to be of little importance when calculating the water balance of the plant. The curves for E/A reflect this as, visually, the curves show a reasonable fit to the data.

Thus no interaction between the effects of D and Q on g_s is evident. For A, however, there appeared to be a decline of *ca* 15% between A at the lowest and the highest value of D, for all levels of Q. This is shown to be significant by the analysis of variance. Although the analysis also shows that there may be an interaction between D and Q in absolute terms (see the slopes in table 5.2), if the decline in A is expressed as a percentage of the value at low D the declines are very similar.

The fact that A consistently declined in the absence of any stomatal closure is hard to explain. Such a marked effect was not found for the experiments reported in Chapter 3. There are several possible explanations.

i) There is a consistent error in the measurement of g_s, or A. Preliminary tests with wet filter paper, instead of leaves, in the chamber showed that the accuracy of measurement of conductance

was within the levels discussed in Chapter 2, over the range of D used in this study, and thus such errors are unlikely to mask a change in g_s of 15%. Tests of the IRGA showed that the largest errors were likely to be caused by zero-drift which, over the two weeks which the experiment took, would introduce random 'noise' in the data rather than a trend with D. Tests for cross-sensitivity of the IRGA to water vapour showed such effects were likely to cause errors of less than 5% in the worst case, which would in any case cause an overestimate of A when the difference between the water vapour concentration in the reference and sample gas lines was maximum, i.e. when D was large. This would act to offset the decline observed.

ii) Diurnal rhythms in the capability to photosynthesise, or feedback inhibition by photosynthates could be causing a decline in A towards the end of the experiment. Although the experiments described in Chapter 4 seemed to show that these problems are not usually likely to occur, the plants used in this experiment might be more sensitive to these factors.

iii) There is a direct effect of E or D on A, which was otherwise masked in the experiments reported in Chapter 3. Such an effect could either be the result of a direct effect of E on A as proposed by Sharkey (1984) - possibly as the result of local drying, or the sharp steps in D could have caused short-term stress (as discussed in Chapter 4) which reduced A.

Unfortunately there are not enough data to distinquish which one or possible combination of these factors might have caused the effect which must therefore remain in question.

Despite the decline of A at high D, the graphs of E/A (fig. 5.4) are still mainly linear, as there was little or no response of g_s to D. The graphs show that the absolute values of E/A, at high Q, are broadly the same as those presented in Chapter 3: as Q was reduced then E/A increases markedly. This is a result of the different shapes of the light response curves for g_s and A (see above). It is hard to say whether this

type of response would be found for plants outside, as it is likely that both response curves for g_s and A would be different for such plants.

The conclusions that can be drawn from this experiment are unfortunately limited, as the stomata failed to show significant sensitivity to D at any level of Q. Thus one can only conclude, for this particular species and shoot age, that sensitivity to D does not increase at levels of Q below the level at which g_s is light saturated. Thus as Ng (1978) worked with similar plants, such an interaction is unlikely to explain the difference between the responses of g_s to D he reported and those in this thesis.

The implications of these responses of both g_s and A to Q are further analysed with respect to stomatal control of E and A in Chapter 10.

CHAPTER 6

ANOMALIES EXPLAINED

6.1 Introduction

One of the original objectives of this project was to investigate further, the extremely strong stomatal response of Scots pine to saturation deficit, reported by Ng (1978). Many of the experiments described in the earlier parts of this thesis, plus many other preliminary experiments, were, in part, done with the aim of reproducing the results of Ng so as to provide a baseline for further investigations. As has been shown in previous chapters, in Scots pine the response of g_s to D varied from no response (in older shoots) to a moderate response in shoots 12-weeks-old. However, in none of the experiments was there any evidence of a decline in E as D increased and there was therefore no basis on which to investigate possible mechanisms for a feedfoward response of stomata to D.

In late 1981 the Model 440, E.G. & G. dewpoint meter broke down. Whilst it was sent away for repair, a Model 880 E.G. & G. dewpoint meter was installed as a substitute. Several experiments thereafter revealed a discrepancy between results obtained from the differential Vaisalas and the 880 meter. Prior to this, the results obtained from the Vaisalas and the 440 meter had shown good agreement. Despite recalibration of both the Vaisalas and the 880, a large discrepancy was still found. Several experiments were then done to quantify the difference. One of these is described below.

In addition, after further investigations of the performance of the 880 dewpoint meter (see below), a computer simulation of an experiment where the response of the stomata of a hypothetical shoot to D is measured was developed. This was done to study the effect of the performance of the 880 on a known response to D.

6.2 Plant Material

As Ng (1978) had previously shown that cut shoots of Scots pine showedvirtually no response to D, it was decided to use such material for these tests as a more realistic control than filter paper (it is also easier to measure the temperature of a pine needle than of wet paper). It was also easier to simulate material which had constant g_s in a model (see below). Shoots were cut from a *ca* 10-year-old Scots pine tree (of unknown origin) from outside the building. The shoots were *ca* 10 months old.

6.3 Experimental details

A shoot was cut early in the morning and brought into the laboratory where it was recut under water in a specially constructed vessel which allowed the shoot to be positioned horizontally in the assimilation chamber. The lights were immediately switched on to give a total Q (from both sides) of *ca* 1100 µmol m^{-2} s^{-1}. D was set to an initial value to 0.6 kPa. After three hours D was increased in steps of approximately 0.25 kPa, with an equilibration time of 100 minutes at each step, before measurements were recorded. A total of 8 steps were imposed. Values of gs and E were calculated from both the differential Vaisalas and the 880 dewpoint meter. For these experiments the sensor head of the dewpoint meter was installed in the heated analysis rack (see Chapter 2), as this had been the practise when 880s had been used in the past. The 'gain' of the 880 was set so that the meter did not oscillate at the highest dewpoint encountered during the experiments.

6.4 Results and Discussion of Measurements

Measurements of g_s and E as functions of D are presented in fig. 6.1 and 6.2, respectively. Results are shown for both systems of measuring water vapour. Measurements with other cut shoots and also from experiments with wet filter paper gave similar results. As the difference between the two measurement systems was readily repeatable, no further

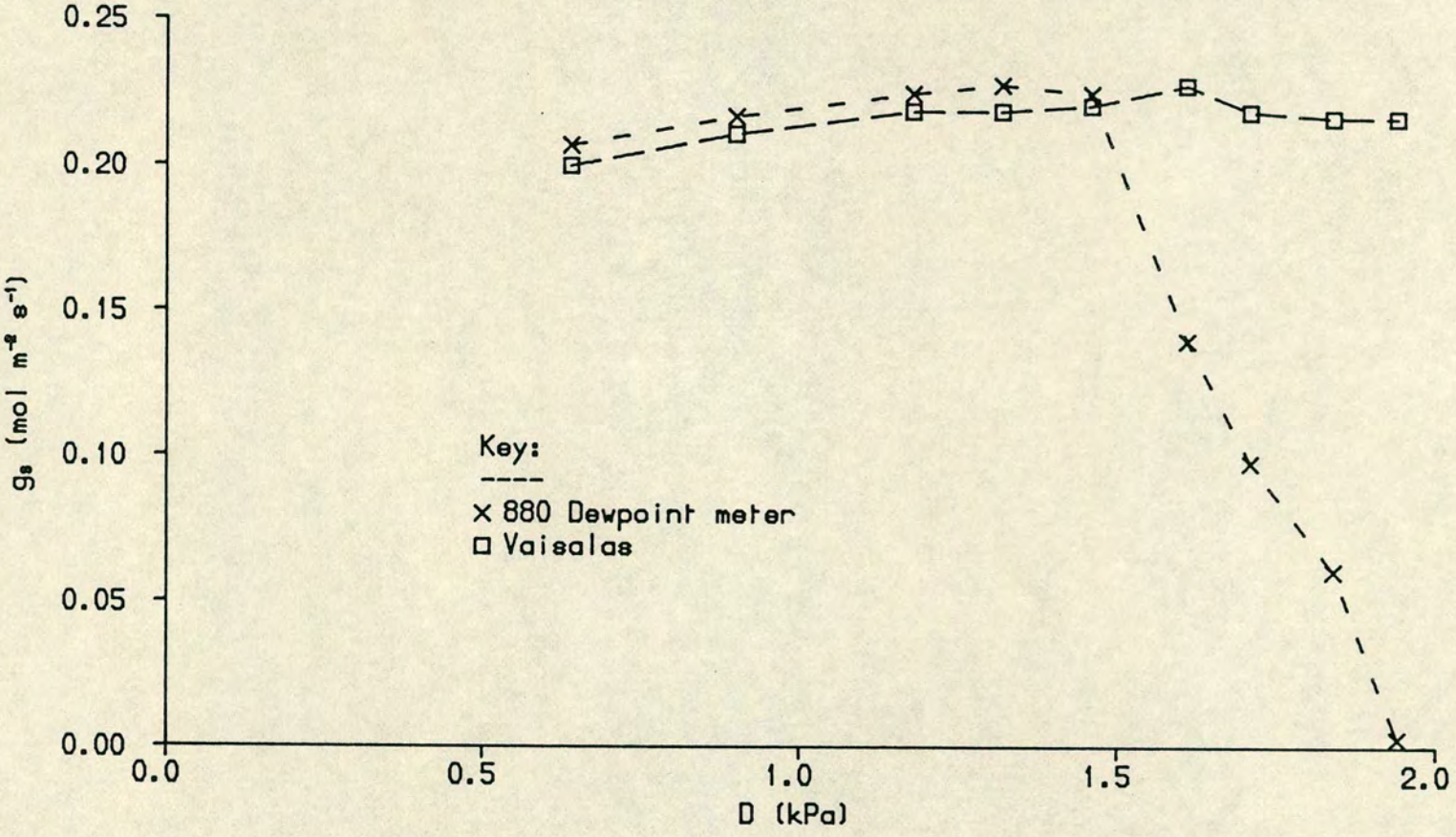

Figure 6.1: g_s as a function of D for a cut shoot, as calculated from the readings of two different water vapour measurement systems. See the key for details.

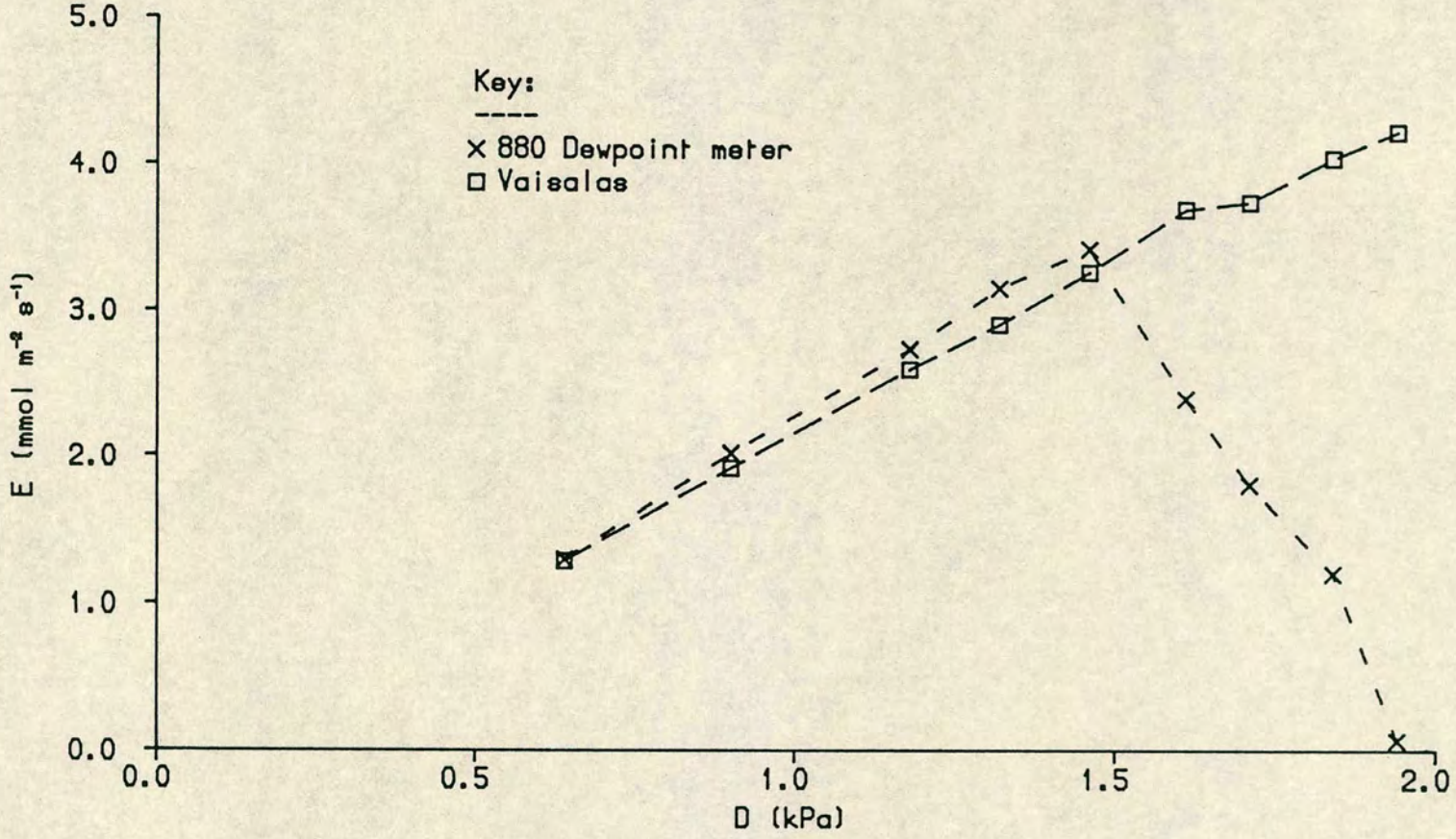

Figure 6.2: E as a function of D for a cut shoot, as calculated from the readings of two different water vapour measurement systems. See the key for details.

data analysis was considered necessary.

The two ways of measuring water vapour gave very different results. The Vaisala system showed that there was no response of g_s to D, i.e. E increased almost linearly. The 880, however showed that E, and thus g_s, declined markedly when D was increased above 1.3 kPa, and became almost zero at 2.0 kPa. The 880 gave a type of response not unlike that reported by Ng (1978) and, at the time when Ng performed those experiments, the 880 dewpoint meters were the only method of measuring water vapour used in the gas analysis system.

As the 440 dewpoint meter had previously been in good agreement with the Vaisalas, the 880 seemed to be at fault. It was removed from the analysis system and tested using an ADC WG-600 water vapour generator to test both its dynamic response and its performance at low dewpoints. These tests revealed that at room temperature the lowest dewpoint that it would measure (when it was supplying the maximum cooling current to the sensor and the 'gain' was set at maximum) was -20 °C, although the instrument has a scale which extends down to -40 °C. Detailed study of the instruction manual revealed that the 880 could, at maximum gain, only cool the mirror to 40 °C below ambient (this is called the dewpoint depression capability), whilst the Model 440 had a stated dewpoint depression capability of -60 °C below ambient. Unfortunately the consequence of this limitation is not clearly stated in the manual for either intrument, and when the meter reaches its lowest limit there is no indication.

The 'gain' setting of the meter was also found to reduce the depression capability. For the 880's used here the potentiometer for setting 'gain' had been brought to the front panel to allow one to increase gain at lower dewpoints. The standard instrument is supplied with a preset 'gain', which is set to prevent oscillations at higher dewpoints. Thus the problems discussed here would be worse if using the standard version, in a similar experiment.

Although a lower limit of -20 °C is not likely to cause serious errors in the laboratory, the dewpoint meters were normally installed in a heated

cabinet at 30-35 °C to avoid the risk of condensation in the air lines. For the 880 and 440 this increased the minimum readable dewpoint to *ca* -9 and -30 °C respectively. Whilst at -30 °C the water vapour pressure is only 0.038 kPa, at -9 °C it is 0.284 kPa. Thus for determination of the dewpoint of very dry air the 880 meter could result in substantial errors.

To produce differences in H_2O concentrations that are large enough to be measured accurately between the air entering and the air leaving the chamber, it was standard practice in this laboratory to use a constant flow of air into the chamber and to vary the dewpoint of this air so as to control D in the chamber. To obtain large D virtually totally dry air entered the chamber and in some instances the flow was increased as well. Although the dewpoint of the air leaving the chamber was unlikely to be less than -9 °C, the dewpoint of the air entering the chamber was often much drier than this (often < -30 °C). Error in measuring the very low dewpoints of the reference air was thus considered to be the possible cause of the erroneous results from the 880 dewpoint meter, as shown in figs. 6.1 and 6.2.

6.5 Simulation

To test this suggestion further and to see if such errors could have influenced the results of Ng, a simple model of the response of the 880 dewpoint meter was written (a listing of the Fortran 77 program is given in Appendix 5). The model simulates an experiment in which the response of g_s to D is measured.

A simplified equation, derived from those given in Chapter 2, for calculating g_s from the gas analysis data is:

$$g_s = \frac{(e_o - e_e)}{D} \cdot \frac{F_e}{L} \qquad 6.1$$

Details of the symbols are in Appendix 1.

For a given leaf temperature, e_0 ($=e_a$) can be calculated from D. Thus, if F_e/L is also given, e_e can be calculated, for any value of g_s.

For the purposes of the model, g_s was assumed to be constant, i.e. there was no response to D, as found for old Scots pine shoots (see Chapters 2 & 5). A typical initial value of 0.4 mol m^{-2} s^{-1} was used for F_e/L. D was increased in steps from 0 to e_i and values of e_0 and e_e calculated for each step. For large values of D, e_e was, in some cases, found to go negative. When this happened e_e was set to 0 and F_e/L was increased from the initial value. This is equivalent to the real situation where the flow would be increased when e_e could be reduced no further (see above).

The derived values of e_0 and e_e were then used to recalculate g_s and E, after the limitations of the 880 had been imposed on the values. A typical lower limit of measurement by the 880 of -9 °C was taken: this is equivalent to a water vapour pressure of 0.284 kPa. If the calculated value of e_a or e_e was less than this, it was fixed to 0.284 kPa as this is the reading that would be recorded in a real experiment.

The results of three runs of the program are presented for g_s and E in figs. 6.3 and 6.4, respectively. The runs represent three different temperatures: 10, 20 and 25 °C . The fixed values of g_s, used at each temperature were taken from Ng's graph (his fig. 4.6.1, 1978). The values of g_s were read, for each temperature, for the smallest values of D at which errors due to the 880 would not occur. The input values of g_s (and the values of E calculated from them) are represented on the graphs by the dashed lines.

All the solid curves, representing the results as measured by the 880, show a drastic decline in g_s and E when D reached a critical value. This value corresponds to the point when e_e fell below the simulated minimum reading for the 880. As D increased further g_s and E fell rapidly to zero. At 20 and 25 °C there was a slight 'kink' in the rate of decline.

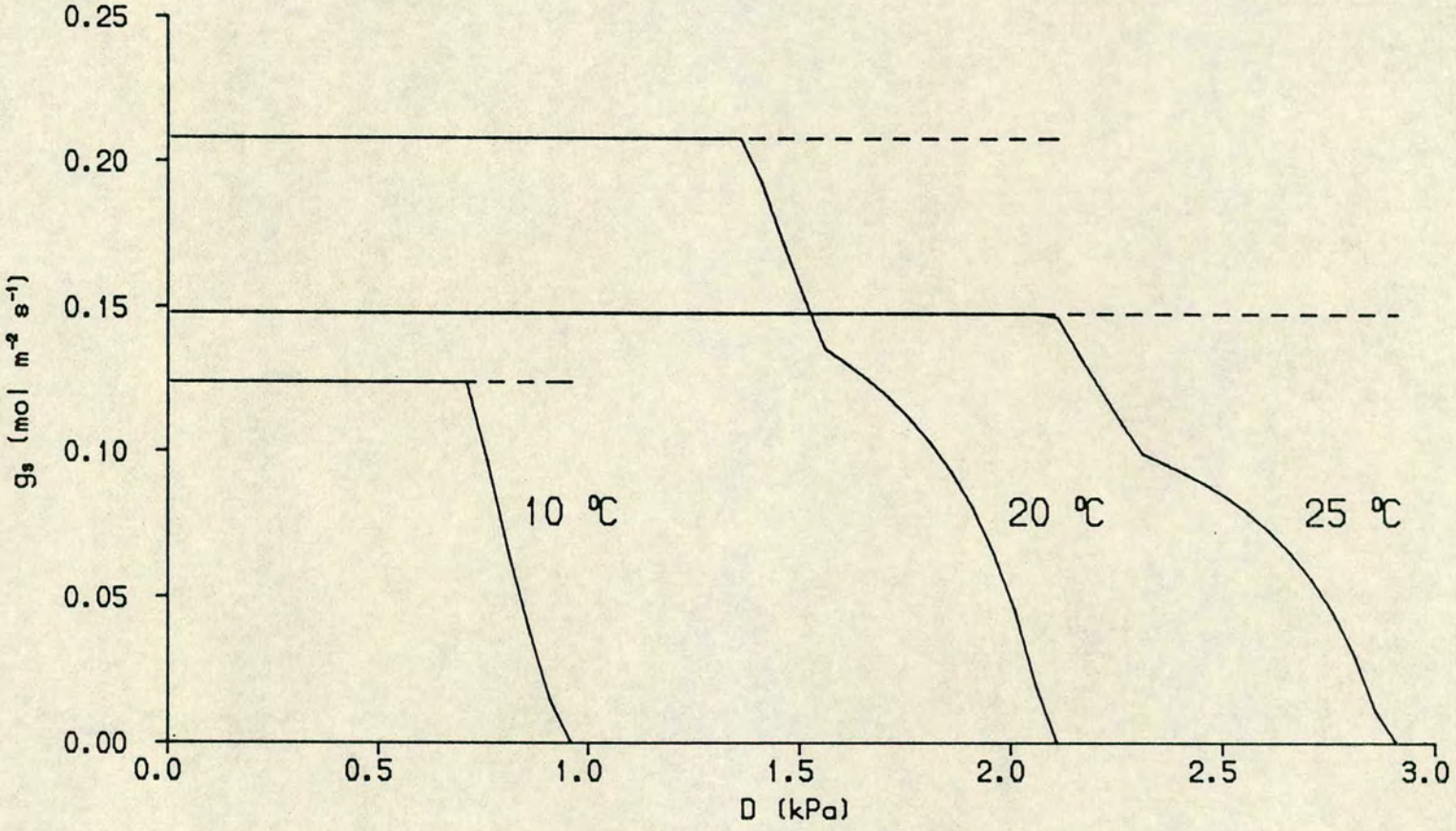

Figure 6.3: The output of a computer simulation of an experiment to measure g_s as a function of D, using the 880 dewpoint meter, for three temperatures. The solid lines represent the output from the 880 meter. The dashed lines represent the input values of g_s.

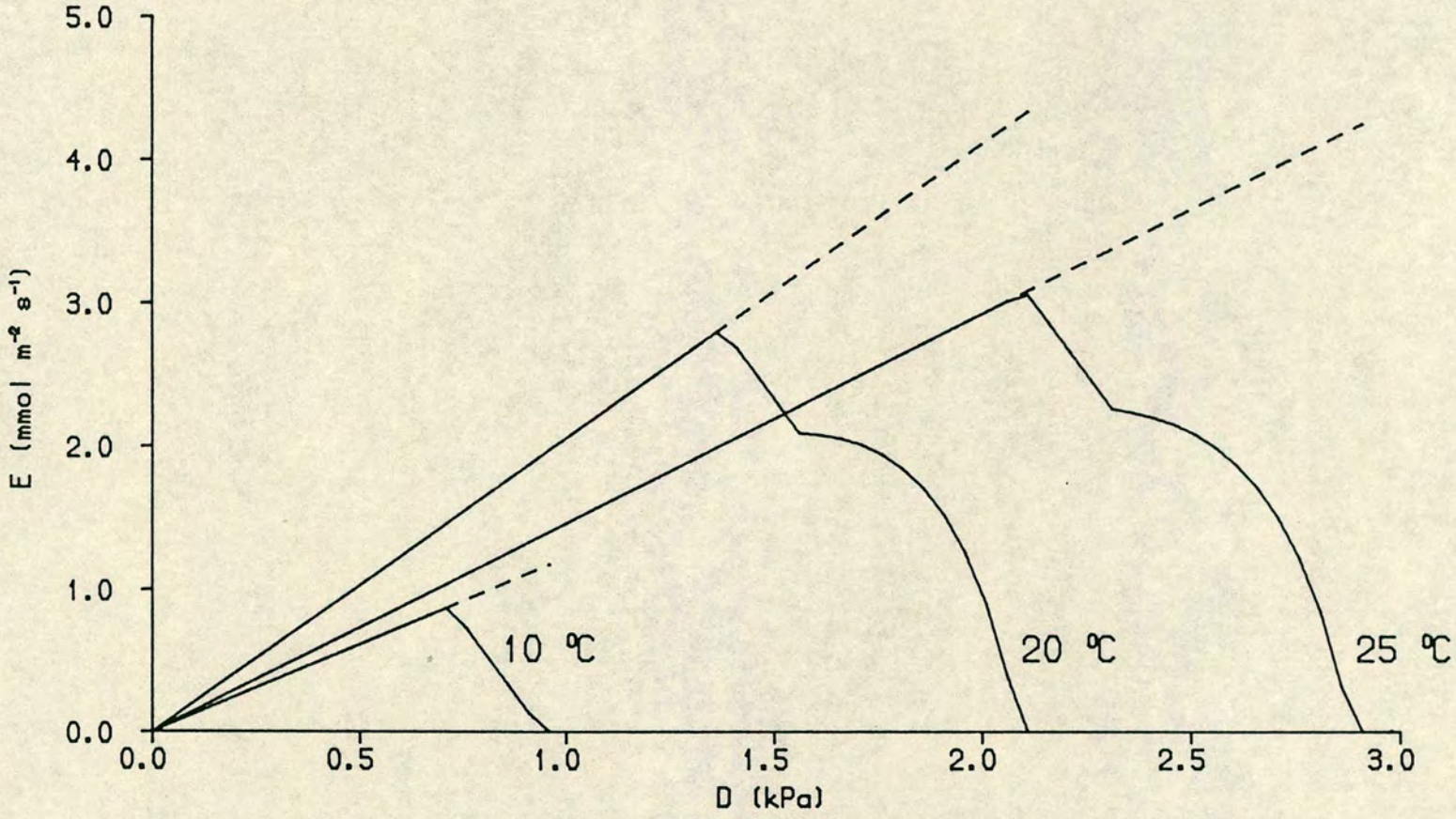

Figure 6.4: The output of a computer simulation of an experiment to measure E as a function of D, using the 880 dewpoint meter, for three temperatures. The solid lines represent the output from the meter, the dashed lines represent the E calculated from the input value of g_s.

This corresponds to the point where the flow had to be increased because the theoretical value of e_e reached 0 kPa.

6.6 Discussion

Over the range of measurement, the results shown in figs. 6.1 and 6.2 are very similar to those produced by the model and shown in fig. 6.3 and 6.4. The simulated 880 response also produced very similar results to those of Ng (1978). Thus it seems likely that Ng's results may well have been artifacts caused by incorrect use of the 880 dewpoint meter. Further evidence that adds weight to this hypothesis, is that measurements of A, made by Ng, which were not originally analysed, show that A remained more or less constant, despite the apparent, almost total stomatal closure as D was increased (pers. comm.). As input to the model of a constant value of g_s generated very similar 'responses' to Ng's, it is likely that g_s was indeed more or less constant in his experiments like some of the results presented in Chapters 3 and 5.

Whether similar experimental problems could be involved in some of the data presented by other workers in the literature is hard to say. These results do however show that measurements of this kind require full understanding of the instrumentation that is used.

The likelihood that Ng's results were probably affected in this way drastically reduces the evidence for conifers exhibiting very strong stomatal responses to D, possibly involving some form of 'feedfoward' mechanism of response. Thus work done after the stage of the project was mainly biased towards understanding the consequences of the stomatal response to D in terms of control of E and A.

CHAPTER 7

INTERACTIONS BETWEEN THE RESPONSES TO TEMPERATURE AND TO LEAF-TO-AIR VAPOUR PRESSURE DIFFERENCE

7.1 Introduction

Much of the work done prior to the experiments described in the previous chapter was done partly to discover if the responses described by Ng (1978) could be reproduced for other species and experimental conditions. One aspect not covered in these experiments was the interaction between the response of g_s to D and the response to temperature (T).

Studies of the responses of stomata to temperature alone have produced quite conflicting results perhaps because in many early experiments D was not controlled as T was changed (see Lösch, 1979b). Even in a recent paper (Farquhar, 1978), theoretical arguments were based on the data of West & Gaff (1976) where D had been varied by changing T.

In general, it is now accepted that, at a constant level of D, g_s will increase with temperature, up to an optimum temperature and then decline. Whether the optimum temperature is reached, or whether the sensitivity to T is large over the normal environmental range of T, is dependent on the species in question and, to a degree, on the growth conditions (Schulze, 1974; Neilson & Jarvis, 1975; Lösch, 1977).

Whilst there have been many studies of the response of g_s to D and to T, there have been relatively few studies of the interaction between the responses to D and T. This is surprising, because if the responses of g_s to D is temperature dependent, there are important implications for the mechanism of the response of g_s to D, i.e. whether 'active' processes are involved (see below). Analysis of the sensitivity of the stomata to D at different temperatures is, however, complicated, as when T changes, A is also likely to change, and for species whose stomata respond to C_i this adds an uncontrolled variable to the experiment.

Hall & Kaufmann (1975a+b) present data for three species - *Helianthus annuus* L., *Sesamum indicum* L. and *Betula vulgaris* L. (See also Hall, Schulze & Lange, 1976 for further data for *S. indicum*.) For all three species the stomata were less responsive to D at higher T. However, A increased with temperature and hence for these species changes in C_i complicate the situation. A similar result was found by Lösch (1977) who worked with isolated epidermis of *Polypodium vulgare* L., in CO_2 free air. He showed that stomatal aperture was less sensitive to D at high rather than low T, even though C_i was forced to 0 µmol mol^{-1}.

Studies of this kind on coniferous species are limited, to my knowledge, to the results of Ng (1978). The computer simulation of the results of Ng (1978), described in the previous Chapter, provides indirect evidence for the absence of an interaction between the response of g_s to T and D in Scots pine. The model, which assumed no response of g_s to D, gave very similar results to those of Ng for all three temperatures, and thus by implication g_s was more or less constant at all three temperatures.

Because of the generally confused picture and the lack of evidence in conifers, an experiment was devised to see if there was an interaction between D and T. I decided to use Sitka spruce for this study as it had previously been shown to have a comparatively strong response of g_s to D. In addition the response of g_s to C_i (Beadle *et al*, 1979; Morison, 1980) and the response of g_s to T, independent of D, (Neilson & Jarvis, 1975) had previously been measured.

7.2 Plant Material

Potted (1+2)-year-old seedlings of Queen Charlotte Island provenance were used. The shoots measured were 12-weeks-old at the start of the experiment. The potting compost and growth conditions were as specified in Chapter 3.

7.3 Experimental details

The pretreatment and experimental procedures were identical to those described in Chapter 3, except that the ranges of temperature and D imposed were different. Three leaf temperatures were imposed: 15, 20 and 25 °C. These values were chosen as they include the preconditioning temperature plus one temperature above and one below. Unfortunately, failure of the air conditioning system in the room which housed the gas-analysis system at this stage in the project, made it impossible to impose higher or lower temperatures than this, without risk of condensation at some point in the system.

The plant, to be measured, was taken from the growth room and the shoot fixed in the assimilation chamber on the evening prior to the day of measurement. The value of T at which the experiment was to be done was set that evening. The overnight level of D was set to approximately mid-range of the values to be imposed at that T. On the day of measurement D was intially decreased to the lowest value, then increased in five steps to the highest value. The lowest value of D was set so that there was no risk of condensation in the chamber. The highest value was limited by the ability to dry the air in the chamber.

Three replicate plants were used. This number of replicates was too small for techniques of randomising the order of the experiments to be valid. The experiments were therefore done in the following order: shoot 1: 20, 15, 25 °C; shoot 2: 25, 20, 15 °C; shoot 3: 15, 20, 25 °C. This order was used to ensure minimal bias in the results if there was any effect on the responses, of the order in which T was imposed on different days.

7.4 Results

The data for the three shoots were standardised to a common value at the lowest D, at 20 °C. The mean values and actual standard errors, for this condition were for g_s, 0.113 (±0.004) mol m^{-2} s^{-1}, for E, 0.720 (±0.139) mmol m^2 s^{-1} and for A, 6.513 (±0.423) µmol m^{-2} s^{-1}. Graphs of

the response of g_s, E and A are presented in figures 7.1, 7.2 and 7.3, respectively. The data points represent the means of the three replicates plus one standard error. The curves were fitted in a way identical to that descibed in Chapter 3, i.e. a rectangular hyperbola fitted to E/D, a transformation of this for the g_s/D curve and a staight line fit for A/D. The fitted parameters for the E/D curves are given in table 7.1 and for the A/D curves in table 7.2. A graph of E/A *versus* D is shown in fig. 7.4. The curves were calculated from the curves fitted to E/D and A/D.

Table 7.1 The parameters derived from fitting hyperbolic curves, of the form of equation 3.1, to the E *versus* D data for three temperatures. The asymtopic standard deviations of the parameters are given in the brackets. T was controlled to within ±0.1 °C. Units for T are °C, for E_m mmol m^{-2} s^{-1} and for a mmol m^{-2} s^{-1} kPa^{-1}. N=18

T	E_m	a
15	0.840 (±0.093)	4.177 (±1.849)
20	1.005 (±0.073)	4.347 (±1.613)
25	1.433 (±0.226)	2.983 (±1.670)

Table 7.2 The parameters derived from fitting a linear regression of A as a function of D, for three different temperatures. ±One standard error is given in brackets. Units for T are °C and for the slope µmol m^{-2} s^{-1} kPa^{-1}. Units for the intercept are as for A, i.e. µmol m^{-2} s^{-1}.

T	Slope	Intercept	r^2
15	-2.167(±0.266)	6.835(±0.250)	0.8133
20	-2.250(±0.262)	8.027(±0.362)	0.8217
25	-1.620(±0.256)	7.834(±0.499)	0.7144

The graphs of g_s and A *versus* D shows that g_s and A increased, over the range of comparable D, as T was increased. As an aid to see if there was any interaction between T and D over this range, the predicted values of g_s and A were calculated from the fitted curves at 0.5, 1.0 and 1.5 kPa for the three temperatures and plotted as a function of T in

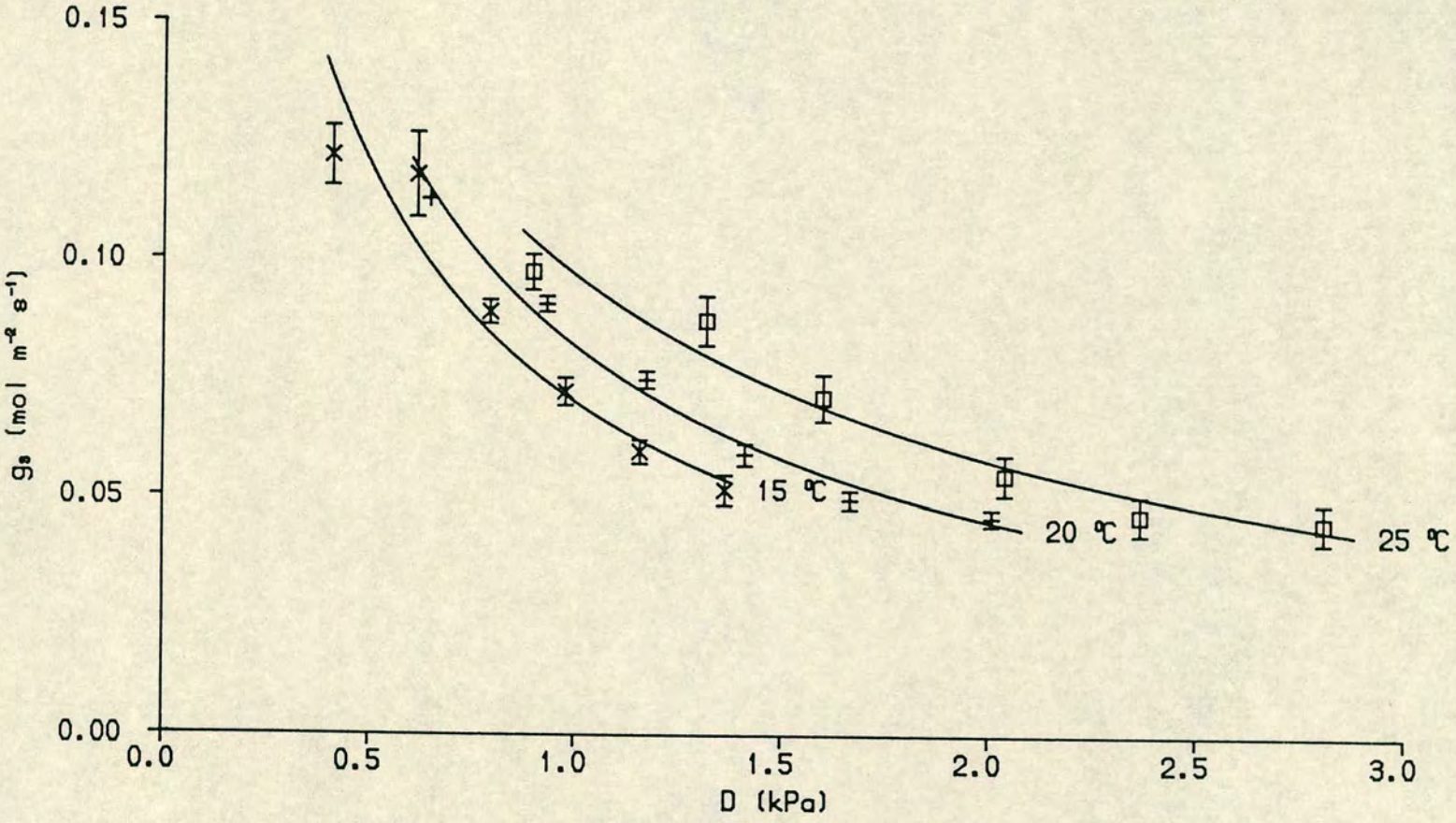

Figure 7.1: g_s as a function of D for three temperatures. Data points represent the mean of 3 replicates, plus 1 S.E. See the text for a description of the fitted curves.

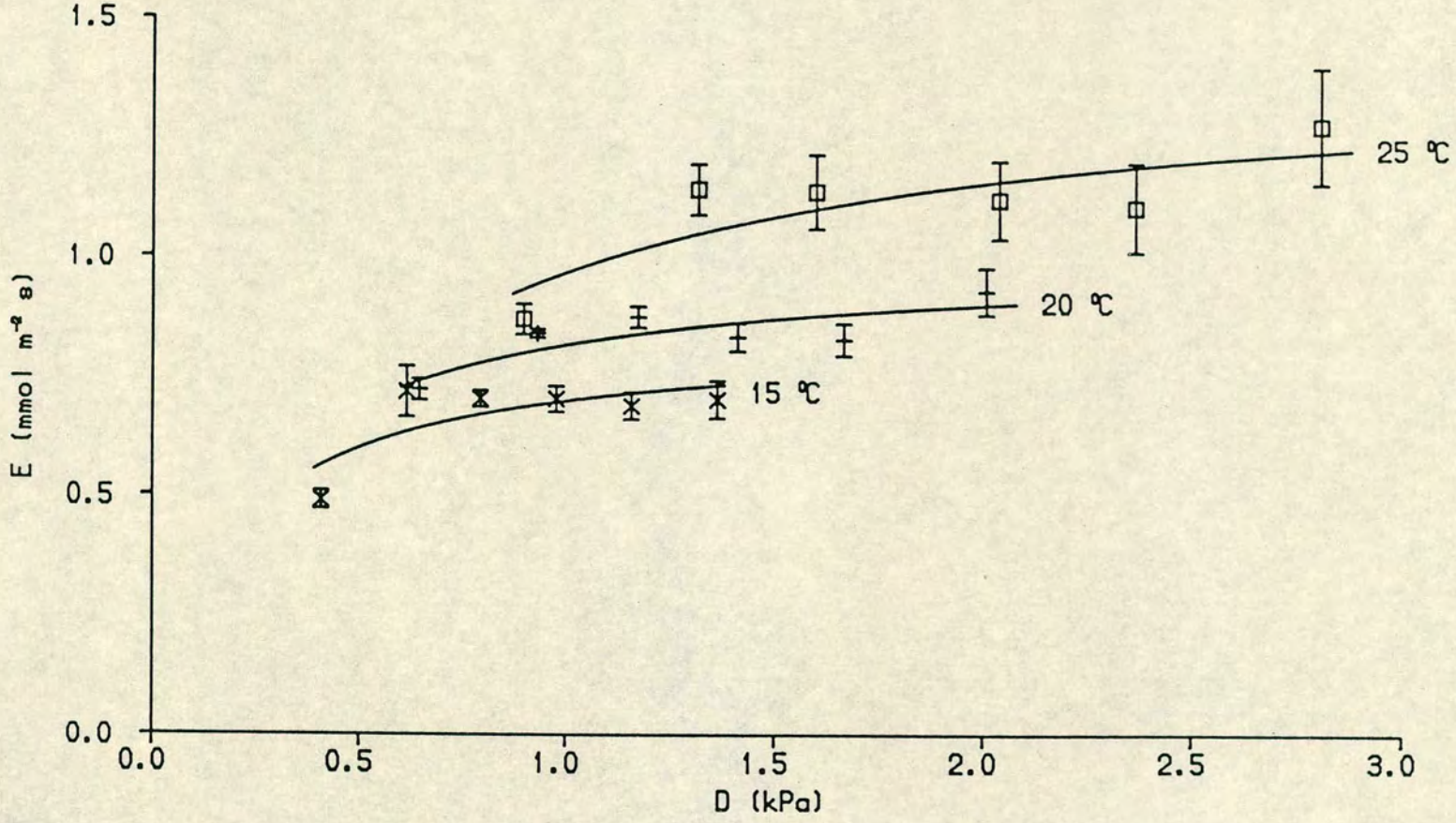

Figure 7.2: E as a function of D at three temperatures. Data points represent the mean of 3 replicates, plus 1 S.E. See the text for a description of the fitted curves.

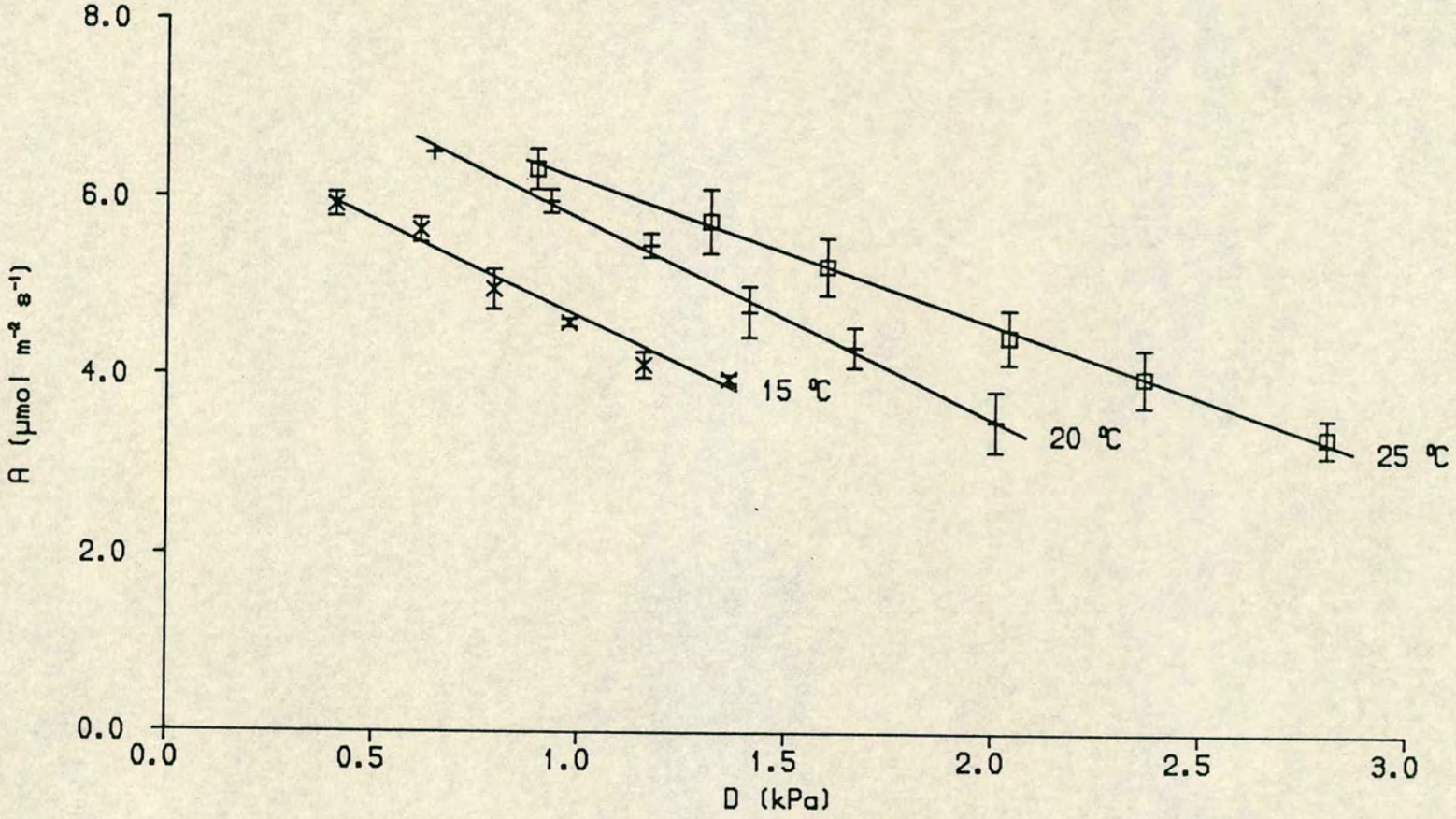

Figure 7.3: A as a function of D for three temperatures. Data points represent the mean of 3 replicates, plus 1 S.E. The lines were fitted by linear regressions.

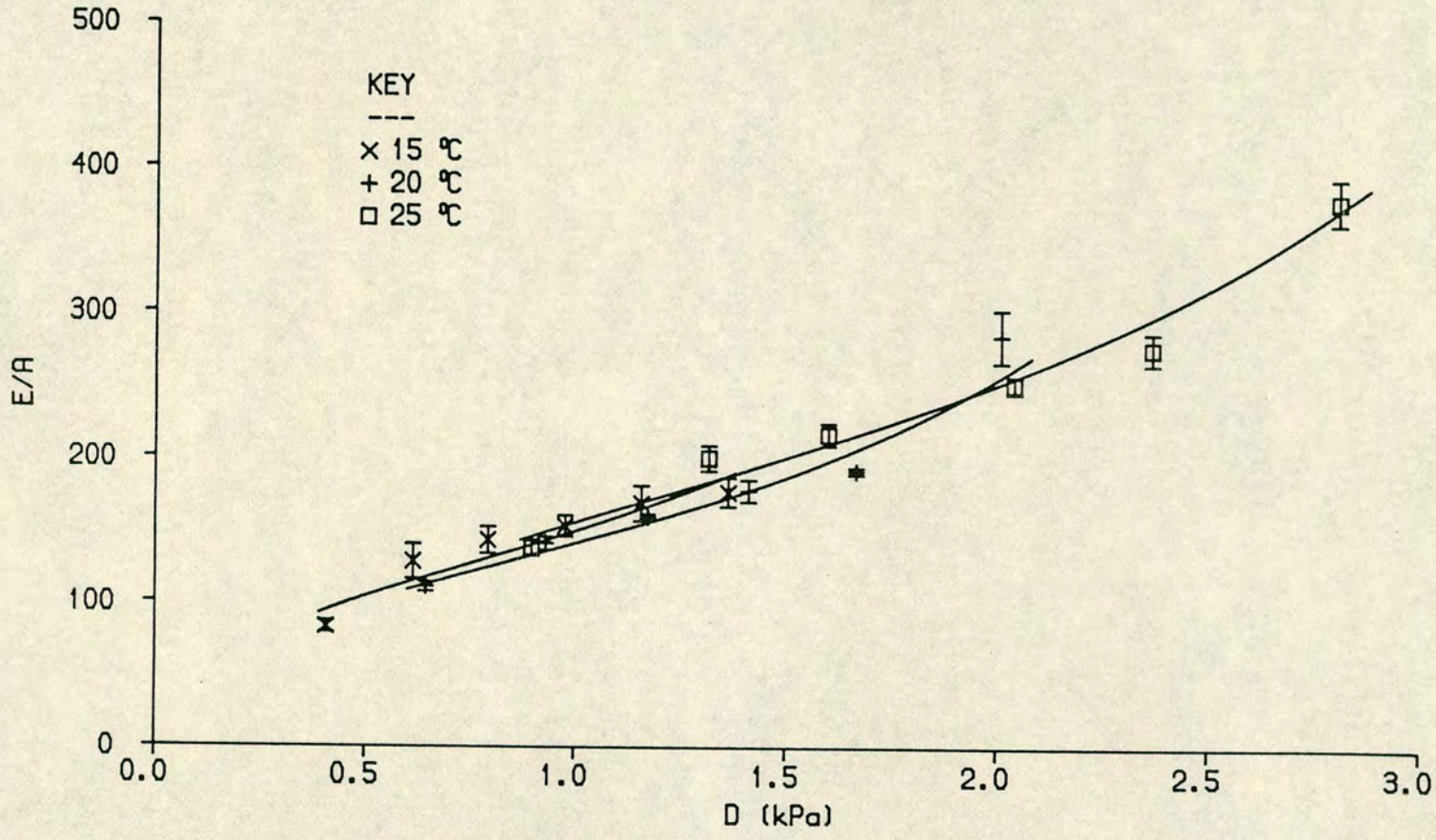

Figure 7.4: E/A as a function of D for three temperatures. Data points represent the mean of 3 replicates, plus 1 S.E. See the text for a description of the fitted curves.

figures 7.5 and 7.6, respectively. These graphs can only be considered as a simple indication as it is hard to assess the errors involved in the fit of the non-linear curves. In addition, the values calculated from the fitted curves are, at 0.5 kPa, extrapolated below the range of measurement for the experiments at 20 and 25 °C, and at 1.5 kPa above the range of measurement for the experiment at 15 °C. Unfortunately with only three replicates and only two or three measurements in the range of comparable D, no valid statistical tests can be applied to these data.

In addition, to show further the trends in absolute sensitivity of g_s to D, at the different T, a graph of dg_s/dD *versus* D is given in fig. 7.7, produced, as in Chapter 3, by differentiating the fitted curve for g_s *versus* D.

As C_i may play an important role in any change in g_s at different T, a graph of C_i *versus* D is shown in fig. 7.8. for the three temperatures. These data were not standardised. Whilst there appears to be no difference in the relationships between C_i and D at different temperatures, i.e. all the data appear to lie on the same line, there is a trend for C_i to decrease with increasing D. To test this a regression analysis was applied to all the C_i/D data (pooling together the different T treatments) the results of which are given in table 7.3.

Table 7.3 A summary of the results of a regression analysis between C_i and D, with the data for all temperatures together. ±One standard error is given in brackets for the slope and intercept. Units for the intercept are μmol mol^{-1} and slope are μmol mol^{-1} kPa^{-1}.

Slope	-25.5	(±4.0)
Intercept	247.3	(±5.9)

t-value for a test for slope ≠ 0 = -6.4018
For N = 54, this is significant for $p < 0.001$

7.5 Discussion

Over the comparable range of D, there was an increase in g_s as T was increased (fig.s 7.1 & 7.5). Therefore the temperature response of g_s

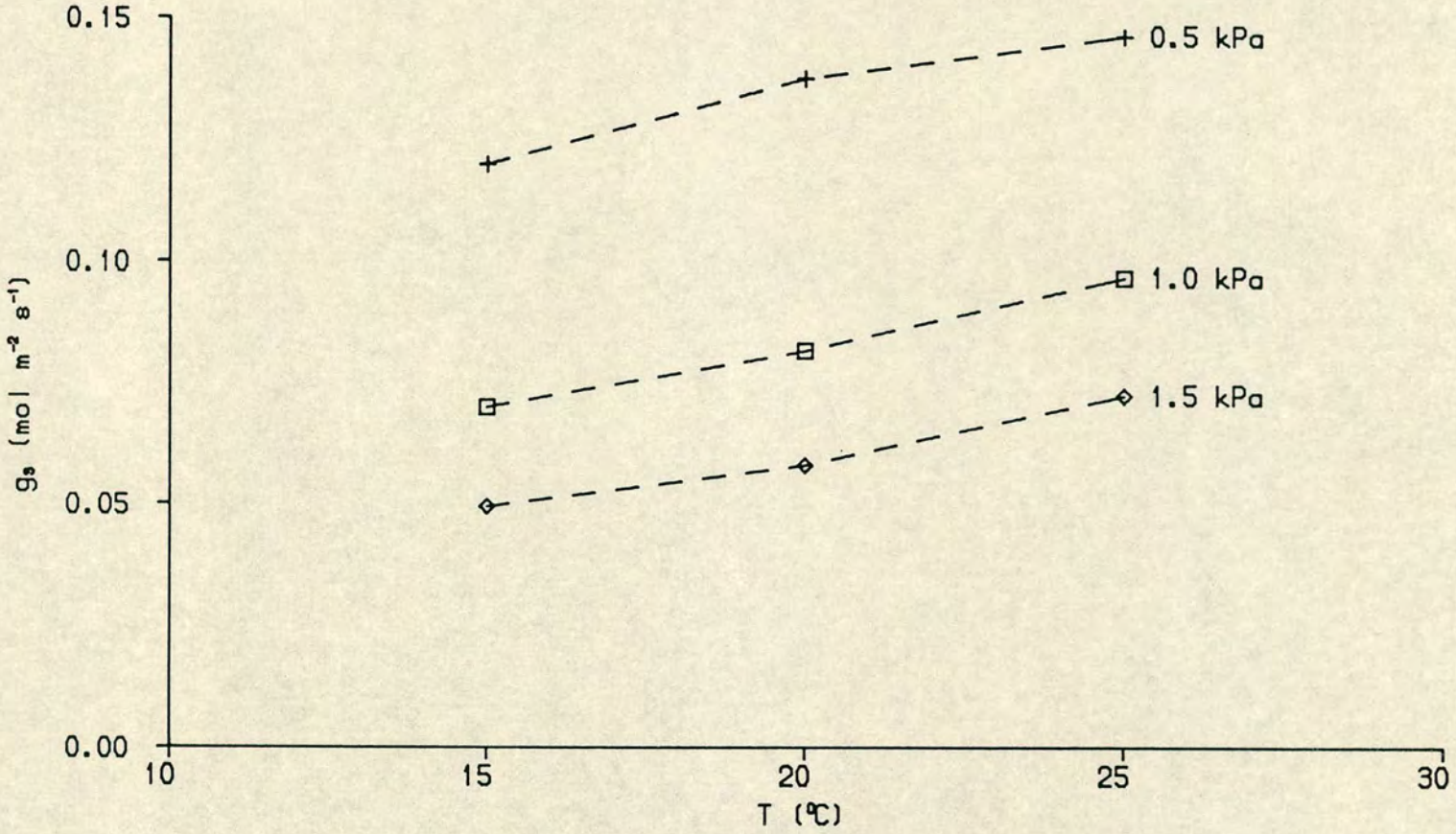

Figure 7.5: g_s as a function of T for three levels of D. The values were calculated from the curves fitted to the data, see the text for details.

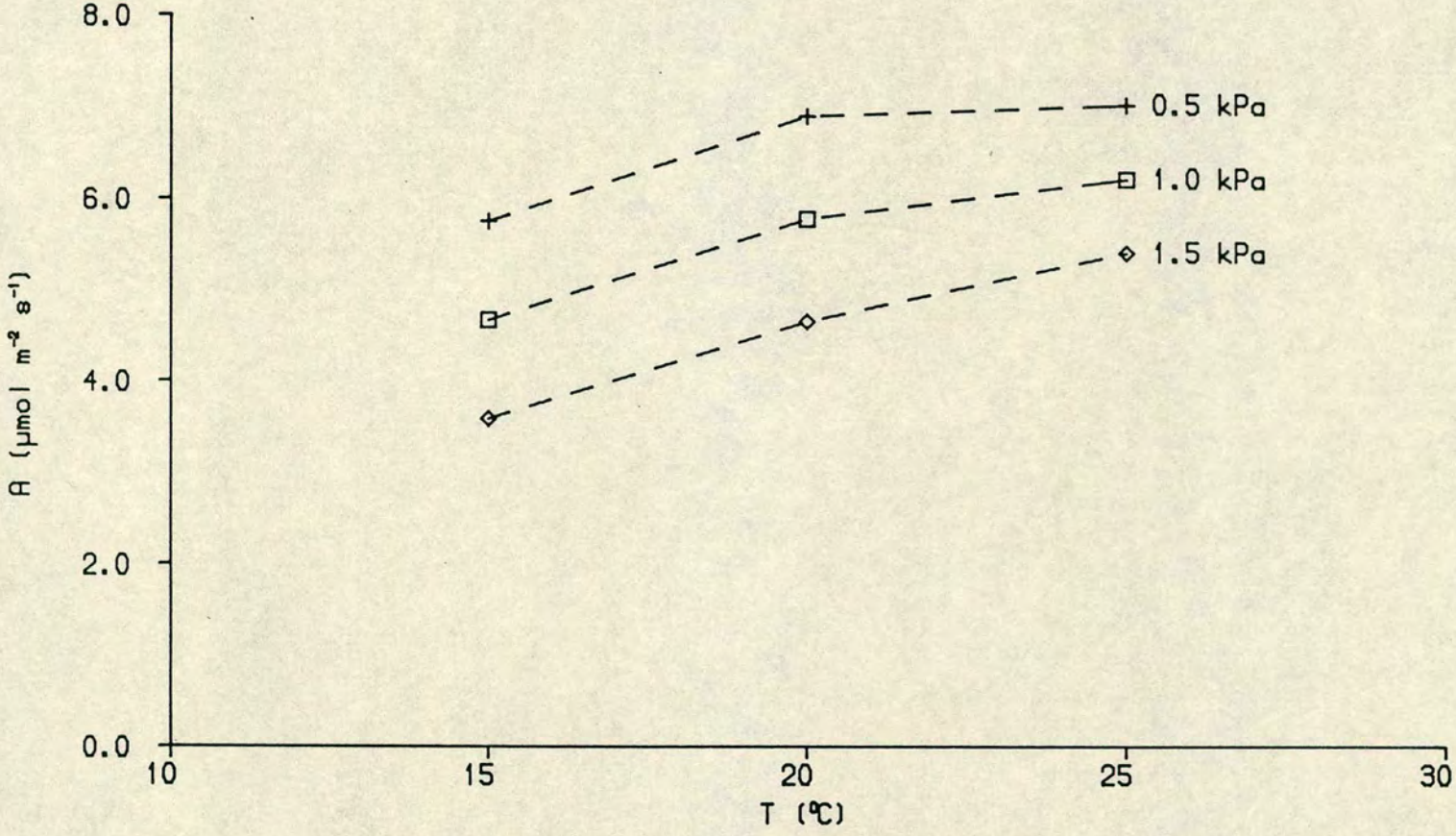

Figure 7.6: A as a function of T for three levels of D. The values were calculated from the curves fitted to the data, see the text for details.

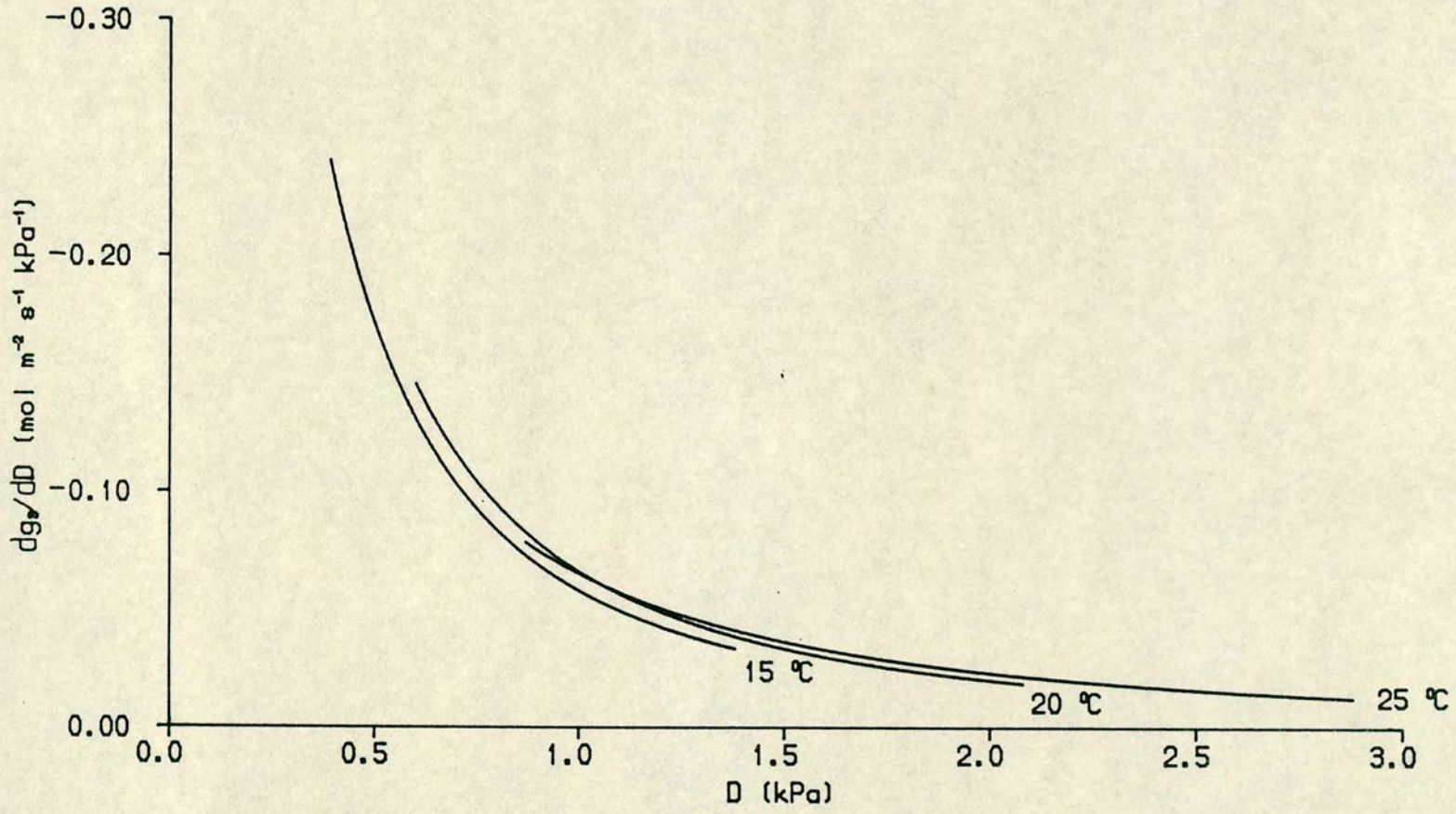

Figure 7.7: dg$_s$/dD as a function of D, for three temperatures. The curves represent transformations of the functions fitted to the E *versus* D data.

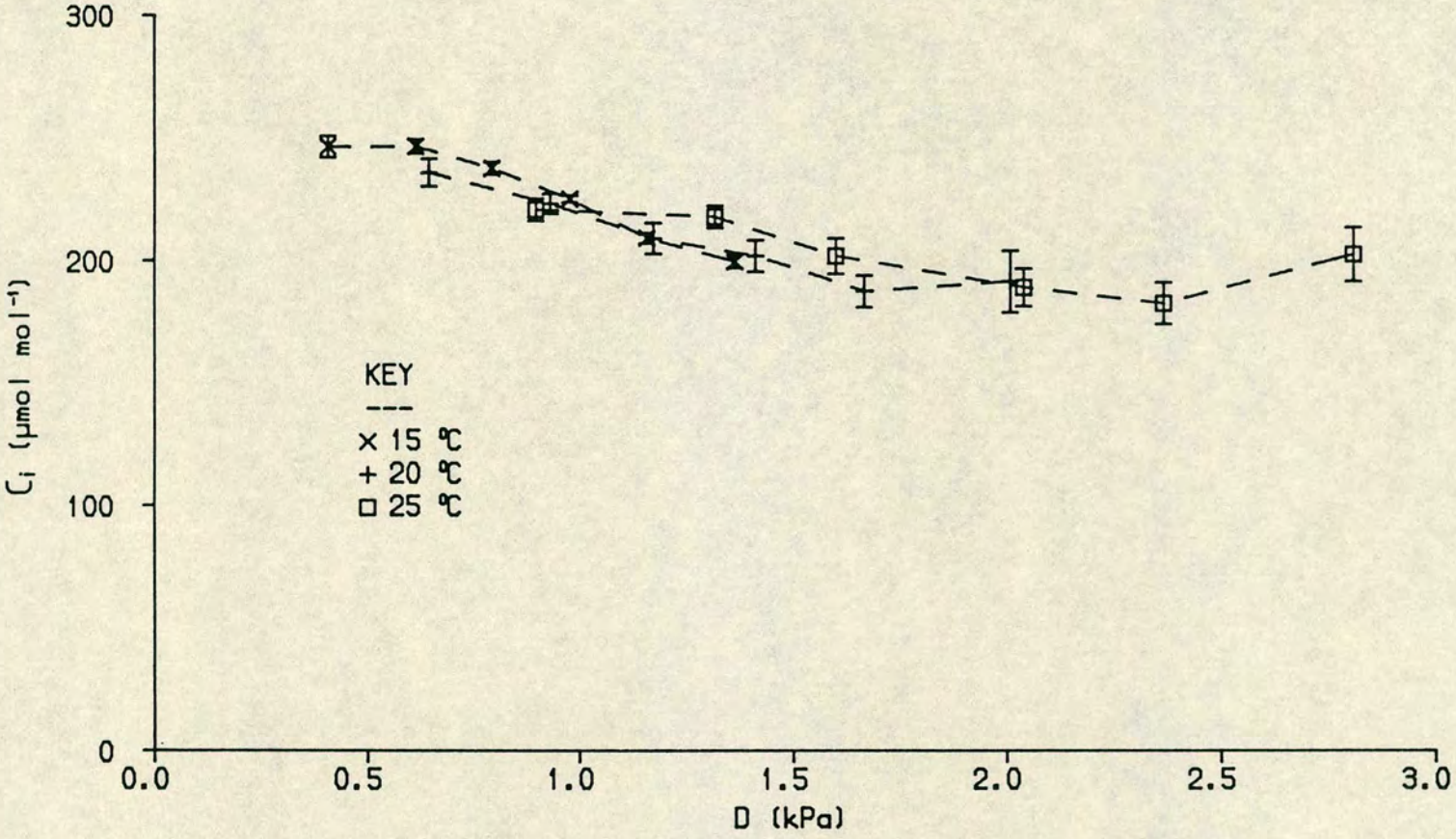

Figure 7.8: C_i as a function of D for three temperatures. Data points represent the mean of 4 replicates, plus 1 S.E. The points are joined by straight lines.

does not appear to have reached the optimum that Neilson & Jarvis (1975) showed for Sitka spruce. The highest T in their growth conditions was only 18/15 °C (day/night) in contrast to the 20/20 °C used in this study, so this may in part explain the difference.

Fig. 7.5 indicates that the sensitivity of g_s to T in absolute terms, is more or less constant, as the lines for the different levels of D are parallel. From this one can imply that the response to D must be independent of T. This is confirmed as, over the same range of D, the curves for g_s *versus* D (fig. 7.1) appear to be parallel. Sensitivity to D (dg_s/dD) appears to be a function of D and to be independent of T, as although the parameters of the fitted curves are different, the curves of dg_s/dD *versus* D (fig. 7.7) overlap in a such a way that they, in practise, represent one continuous curve. Clearly there is no evidence for the degree of interaction as reported by Hall & Kaufmann (1975a+b) for *S. indicum*.

Whilst these graphs indicate that, in absolute terms, there was no interaction between the responses of g_s to T and D, to refer to Chapter 5, the argument of what is defined as an interaction between two variables must again be considered. Considering the predicted values of g_s in fig. 7.5, at 15 °C an increase in D from 0.5 to 1.5 kPa caused a 59% reduction in g_s, whilst at 25 °C a similar increase in D caused only a 50% reduction in g_s. Thus in proportional terms there is some indication of a slight effect of T on the response of g_s to D. Whether this is significant or not, either in statistical terms or with respect to the plant's water use, would require further study to say.

It is clear, that at all temperatures, A declined as D was increased (fig. 7.3), but for different temperatures, A increased with T at the same level of D. Analysis of the responses of A to D and T (fig. 7.6) is complicated because A itself may be temperature-dependent, as well as being affected by the changes in g_s.

Although there is no clear difference in the relationship between C_i and D at the different temperatures (fig. 7.8). The C_i *versus* D curves for the pooled data show that there is a highly significant decline in C_i

as D increased (table 7.3). This decline was a result of the stomata closing as D was increased, which in turn acted to reduce C_i, thereby limiting A (fig. 7.3). As, A increased at the higher temperatures, despite a trend for C_i to decline, A must also have been increasing with T, independent of changes in g_s. It is, however, impossible to determine critically the temperature sensitivity of A, or the degree of stomatal limitation of A (Farquhar & Sharkey, 1982), without knowledge of the A/C_i response curve, at each temperature.

As a result of the increase in both g_s and A, with increasing temperature, the plot of E/A *versus* D (fig. 7.4) shows that the data for different temperatures all lie on the same, almost straight, line; g_s and A apparently increasing in a coordinated manner. Similar results have also been reported for *Prunus armeniaca* by Schulze *et al* (1975b), although they showed that the absolute values of E/A and the slope of the relationship with D was found to change with the time of year. The relationship between E/A and D is also reflected in the almost constant value of C_i at different temperatures, for comparable levels of D (see above).

Whether the responses of g_s and A to T were a result of the plant 'acting to maintain constant C_i' or whether constant C_i was simply a coincidence is open to speculation (Wong *et al*, 1979). However, for these plants, C_i is unlikely to be an important 'driving-variable' in the response of g_s to D or T. This is because the change in C_i was small, C_i only declined by approximately 25 µmol mol^{-1} kPa^{-1} when D was increased, and not at all for increases in T at the same D. Previous workers have reported only small changes in g_s with C_i for this species (Beadle *et al*, 1979; Morison, 1980). Morison (1980), who reported the strongest responses, showed that over the range of C_i of 100-280 µmol mol^{-1} g_s declined by only *ca* 27 mmol m^{-2} s^{-1} per 100 µmol mol^{-1} increase in C_i, in a fairly linear manner. Thus the small changes in C_i were only likely to have a small effect on g_s.

To test critically if C_i plays an important role in the response of g_s to D, one could measure the response of g_s to D whilst manipulating C_a to control C_i at a constant level (initially one could simply use CO_2 free

air). As the role of C_i was likely to be small for Sitka spruce it was not considered necessary to do this, but this would be an important experiment to do for species with much stronger stomatal responses to C_i.

Whether the apparent consistency of the E/A curve, at different temperatures, is of adaptive significance to the plant, i.e. the stomata have adapted to respond in this way as it is beneficial in the field is hard to tell. A test of whether this type of response fits into the mathematical concepts of stomatal control (Cowan, 1977a+b) is discussed in Chapter 10. However, to test fully these concepts an experiment should really be done over a wider range of temperatures, covering the range this species would normally encounter in the field.

CHAPTER 8

INTERACTIONS BETWEEN THE RESPONSES TO WATER POTENTIAL AND LEAF-TO-AIR VAPOUR PRESSURE DIFFERENCE

8.1 Introduction

As was discussed in Chapters 1 and 3, the response of stomata to D was originally thought to be simply a response of g_s to changes in bulk leaf water potential which occurred as a result of changes in E. Results such as those of Schulze *et al* (1972) and those presented in Chapter 3 of this thesis showed that there was a response to D which was independent of bulk leaf water potential. This leads to the question of whether the response of g_s to D is totally independent of bulk water potential or whether bulk water potential may moderate the response in some way. Understanding the interactions between these two variables is necessary when trying to determine the water relations of the guard cells and the likely mechanism for stomatal response; see Jarvis (1980) and Chapter 11.

As in Chapter 3, the reports in the literature of the response of g_s to D at different levels of water stress can be divided into those studies performed in the field and those done in the laboratory. As discussed previously, many of the field experiments suffer from inadequate isolation of the effects of changes in other environmental variables and, in particular, the fact that water potential is often negatively correlated with D.

Field studies of conifers in which both water potential and D have been isolated as independent variables are comparatively few. Several papers have been published by Tan and various co-workers (Tan & Black, 1976; Tan, Black & Nnyamah, 1977) for Douglas-fir where the effects of D and water potential were isolated. However, their analyses were mainly with respect to soil water potentials and were therefore not directly relevant to the interpretation of stomatal responses.

The study by Running (1980) on lodgepole pine did involve some analysis to separate the effects of D and water potential, but the analysis divided the predawn water potentials into only two divisions, and used daily, maximum values of g_s, pooled for a whole season. The physiological significance of these results is thus hard to determine, but the results showed some indication of a stronger response of g_s to D at lower water potentials.

For non-coniferous species, the number of studies in the field, are also limited. Schulze *et al* (1972) (*Prunus armeniaca* L., *Hammada scoparia* (Pomel.) Iljin & *Zygophyllum dumosum* Boiss.) and Schulze *et al* (1975) (*P. armeniaca*) showed that, for all the species they looked at, there was some indication for increased sensitivity of g_s to D. However, their data analysis techniques were rather crude and also possibly distorted by the use of resistances, rather than conductances. The authors of the latter paper stated that laboratory studies would be required to test the findings thoroughly.

In contrast Sterne *et al* (1977) (*Persea americana* Mill. cv. Bacon) showed little or no change in sensitivity of g_s to D at lower water potentials. Some workers have also presented field data that show less of a response of g_s to D at low water potentials, e.g. Ludlow (1980) (*Panicum maximum*). In fact Ludlow states that this type of response is 'by far the most common'.

Laboratory studies for conifers are, as far as could be ascertained, limited to the porometer studies of Engelmann spruce by Kaufmann (1976, 1979), of Douglas-fir by Johnson & Ferrell (1983) and of Scots pine by Ng (1978). Kaufmann (1976) showed virtually the same response to D at low water potentials as at higher potentials, but the conductances for both sets of data are extremely small and the porometer he used may have been subject to large errors (see Chapter 3). In contrast, Kaufmann (1979) showed less sensitivity to D at lower potentials, though again the conductances were very small.

The results of Johnson & Ferrell (1983) showed that the sensitivity to changes in D, away from the pretreatment growth conditions, increased at intermediate water potentials (-0.7 to -2.0 MPa), but the values of g_s converged at lower and higher potentials. At all water potentials, they found an optimum level of D for stomatal opening, which coincided with the ambient growth conditions, with g_s declining at lower and higher values of D. These data are, however, based on very rapid porometer measurements which may have been subject to large errors (see Chapter 3).

Ng (1978) did a very simple experiment, using a porometer, where he compared the response, to daily steps of D, of shoots of potted seedlings and of cut-shoots standing in water. The water potential of the shoots of the potted plants was *ca* -0.7 MPa and the cut-shoots was -0.2 MPa. The stomata of the cut-shoots showed no indication of any response to D, whilst there was a response for the potted plants, implying greater sensitivity to D at lower water potentials. However, it is likely that cutting the shoots and leaving them in water for five days may have changed the responses of the stomata.

Laboratory studies for non-coniferous species are more numerous, probably because of the implications of any interaction on the understanding of stomatal response to D. Raschke & Kuhl (1969) for *Zea mays* L. found no response to D, though they did find rapid responses to changes in water potential imposed by introducing different osmotica into the water supplying the leaf. They concluded that the response to D was simply a response to changes in leaf water status.

Lawlor & Lake (1976) presented drying experiments on *Trifolium repens* L., *Lolium perenne* L. and *Lysimachia nummularia* L. In these experiments the stomata, for all three species, showed a linear relationship between g_s and water potential. In 'humid' air g_s was larger than in 'dry' air at high water potentials. At low water potentials the values of g_s at the two humidities converged. Thus the sensitivity of the stomata to D was less strong, in absolute terms, at the lower water potentials.

Lösch (1979b) working with epidermal strips of *Polypodium vulgare* simulated changes of water potential by changing the humidity of the air below the strips. He showed that there was a linear response between aperture and D. There appeared to be little change in the slope of the response, but the absolute values of aperture were shifted down to a lower level as water potential was decreased. However, it is hard to compare these results with *in vivo* experiments because of problems in translating the measured apertures to equivalent conductances. It is also hard to tell whether his experiment realistically reproduced the *in vitro* water relations of the epidermis.

Using elaborate gas-exhange techniques Schulze & Kuppers (1979) studied the response of g_s of an individual leaf of *Corylus avellana* L. to rapid changes in water potential induced by changing D around the bulk of the plant. They also studied the response to long-term drying cycles. They concluded that there was no correlation between short-term changes in water potential and g_s, but these measurements were done at potentials which they claimed for their long-term experiment to be above a level at which the stomata were found to close. Their results did, however, show that changes in bulk water potential were unlikely to play an important role in the response of g_s to D.

For the long-term experiment they concluded that the response to D was independent of water potential. However, they only presented data for water potentials in the range of -1.8 to -2.5 MPa. Their upper limit is below that found to cause stomatal closure in many other species. At the lower limit the stomata were still open to 30% of the values at -1.5 MPa. Secondly, there was no statistical analysis to justify their conclusions and the slopes of the response to D visually appear to become more positive as water potential declines. (See also Farquhar *et al* (1980b), for further analysis of the same data.)

Hall & Schulze (1980), using the same equipment as for the experiments of Schulze & Kuppers, reported similar results for *Vigna unguiculata* L.. Unfortunately they could not make direct measurements of water potential, but showed that as soil water was depleted the sensitivity of

g_s to D declined, in absolute terms, though in propotional terms the shape of response was similar.

Osunubi & Davies (1980) presented data for *Betula pendula* Roth. and *Gmelina arborea* L., for responses of g_s to D at high and mild levels of water potential. *B. pendula* showed only a slight response to D at high potentials, but an indication for the response to increase when mildly stressed. *G. arborea* showed little response to D at either water potential.

Only the last three studies above presented detailed analysis of the changes in A with D and water potential. Schulze & Kuppers (1979) showed A to decline more or less linearly with D. The slope of this response got less negative at lower water potentials. However, the analysis of this response was complicated by evidence for a direct effect of water potential on A, indicated by a decline of the A/g_s relationship at lower potentials. Hall & Schulze (1980) showed that A also declined linearly with D, with little change in the slope with water potential, however, they did not test if there was any direct effect of water stress on A. Osunubi & Davies (1980) showed that A declined only slightly with increasing D and that there was no change in the response to D at the lower water potentials for either of the species that they studied.

As the results of these studies are somewhat conflicting, I decided to investigate the possiblity of an interaction between the responses to D and water potential for two species of conifers - Scots pine and Sitka spruce. Scots pine was studied to add to the information already collected on other stomatal responses for this species in this laboratory. Sitka spruce was studied as it had been shown to have the strongest response to D in the experiments reported in Chapter 3.

8.2 Plant material

The first set of experiments used (1+2)-year-old Scots pine seedlings of NT10 provenance. The shoots studied broke bud, under 'natural' conditions outside, five months before the start of the experiment.

Three separate shoots were studied, each on a different plant.

The second set of experiments used (1+2)-year-old Sitka spruce seedlings of Queen Charlotte Island provenance. These plants were brought into a greenhouse with extended daylength (16 hours) during late February to induce early bud break. These shoots broke bud nine weeks before the start of the experiment. Four replicate shoots were used for these experiments.

The potting medium and pretreatment conditions were identical to those described in Chapter 3.

8.3 Experimental details

The response to D was measured using an identical procedure to that described in Chapter 3. D was increased from an initial value of *ca* 0.5 kPa in five steps to *ca* 2.0 kPa during the course of a day.

The water stress treatments were imposed by ceasing the watering of the plants and then measuring the responses of g_s and A to D at fixed intervals as the water potential declined. Thus each replicate was measured every four days in the case of Scots pine and every five days in the case of Sitka spruce. When the water potential had reached a preset minimum, the plants were rewatered and then the response to D determined after a further four/five days.

The water potential of the plant being studied was measured when the lights were switched on in the morning and also at the end of the day. As the shoots being studied were to be used for several experiments, needles or side shoots were removed in the morning from another shoot similar to that in the chamber. At the end of the day the sample was taken from a part of the shoot being studied which was not in the assimilation chamber. The water potential of three individual needles were determined in the case of Scots pine and of single small side shoots for Sitka spruce (see Chapter 2).

8.4 Techniques of data analysis and results

Analysis of the results of these experiments is complicated. Firstly, the responses of g_s and A to D were measured at different water potentials for the different replicates. Secondly, it was found for both species that the decline of water potential followed a step-like function with respect to time, i.e. initially there was a slow fall in plant water potential followed by a rapid drop, over three to four days, after which water potential declined slowly. This was partly a result of the type of peat-based potting compost used. As the measurements were done at regular intervals this resulted in there being very few measurements at intermediate levels of stress.

As a result of these two effects, presentation of mean responses for the replicates is very difficult. I have decided, therefore, to present the data by the use of a descriptive model for which parameters have been derived using a non-linear regression program (BMDP, PAR - see Appendix 4).

As the form of the model can influence the interpretation of data such as these, one would like to be able to justify the choice of model by initially testing the data for interactions between variables, using a statistical analysis. However, because of the problems of applying such analyses discussed in Chapter 2, and in addition, because of the uncontrolled nature of the water potential treatments, applying such an analysis was considered totally invalid. I therefore assumed that the responses to D and water potential do not interact (Jarvis, 1976).

The same function, as has been used in the previous chapters (equation 3.3), was used to describe g_s as a function of D. As shown in Chapter 3, this relationship can be expressed as a function of the maximum conductance at D=0 (see equation 3.4).

To descibe g_s as a function of water potential the following relationship was used:

$$g_s = \frac{g_{max}}{(1 + (\psi_{xyl}/b)^c)} \qquad 8.1$$

This function was described by Landsberg (1977) in relation to plant growth. More recently Jones (1983) has used this function to describe stomatal responses to water potential. The function has the property that g_s equals g_{max} when $\psi_{xyl} = 0$: when $\psi_{xyl} = b$ then $g_s = 0.5g_{max}$. The curve can vary between highly sigmoid to a virtual straight line, and can, therefore, cover the two extremes of response reported in the literature, from a sharp threshold to a linear decline of g_s as water potential declines.

As both functions share g_{max} as a common parameter, multiplication of the two functions (3.3&8.1) describes the response to both variables together (Jarvis, 1976). Thus the relationship is:

$$g_s = \frac{P\,a}{1000} \cdot \frac{E_m}{(E_m + a\,D)} \cdot \frac{1}{(1 + (\psi_{xyl}/b)^c)} \qquad 8.2$$

This function was fitted to the standardised data for all the replicates, for each species, using the non-linear fitting program. The reference treatment for the process of standardisation (see Chapter 2) was the lowest value of D, at the highest water potential. The mean values for g_s, E and A in the reference treatment, for the two species, are given in table 8.1. The parameters derived by the curve-fitting program are given in table 8.2.

Table 8.1 A summary of the unstandardised means for g_s and A. ±One standard error of the mean is given in brackets.

i) Scots pine
At the lowest D, and highest water potential the unstandardised mean of -

a) g_s was 0.356 (±0.042) mol $m^{-2} s^{-1}$
b) A^s was 11.86 (±0.541) µmol $m^{-2} s^{-1}$

ii) Sitka spruce
At the lowest D and highest water potential, the unstandardised mean of -

a) g_s was 0.098 (±0.011) mol $m^{-2} s^{-1}$
b) A^s was 6.97 (±0.908) µmol $m^{-2} s^{-1}$

Table 8.2 The parameters derived from a model (see the text) fitted to the g_s data. Units for a are µmol m^{-2} s^{-1} kPa^{-1}, for E_m, mmol m^{-2} s^{-1}, for b, MPa and c is dimensionless. The asymptotic standard deviations of the parameters are given in brackets.

Species	Parameters				No. of
	a	E_m	b	c	measurements
Scots pine	3.946 (±0.132)	8.897 (±0.695)	-1.180 (±0.013)	6.897 (±0.408)	90
Sitka spruce	1.339 (±0.092)	2.271 (±0.244)	-0.985 (±0.014)	6.947 (±0.785)	102

The fitted relationships for g_s are plotted as a function of water potential in figures 8.1a+b, for Scots pine and Sitka spruce, respectively. Six levels of D are shown, each corresponding to the mean value of D imposed in steps during the experiment. The individual data points are also plotted on these graphs to show the fit of the model to the data.

The model fitted to g_s, was transformed to give E as a function of D and water potential, using an approximation of equation 2.5, i.e.

$$E = g_s D / P \qquad 8.3$$

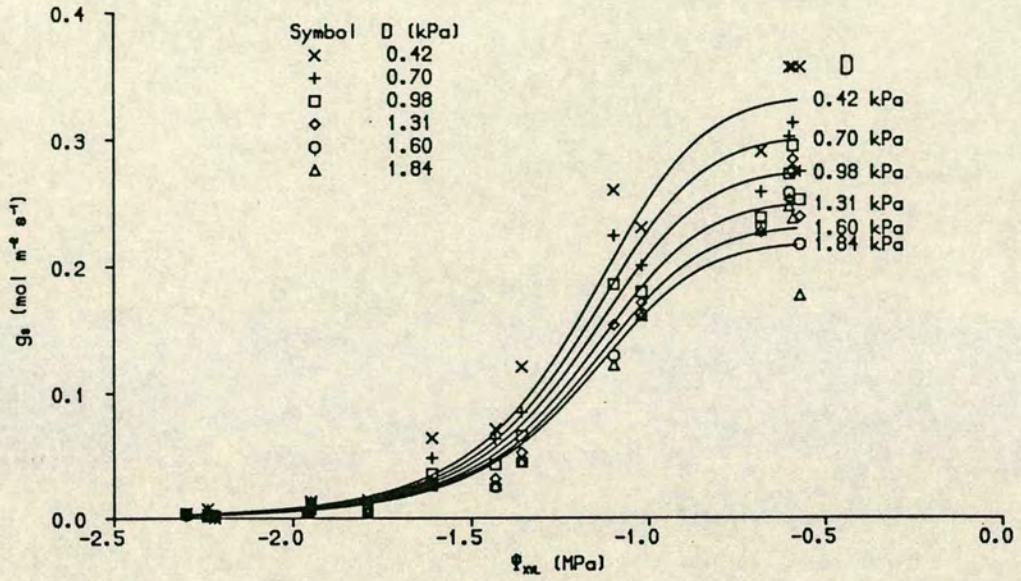

Figure 8.1a: g_s as a function of water potential for Scots pine at 6 levels of D. The data points are for 3 replicates. See the text for a description of the fitted curves.

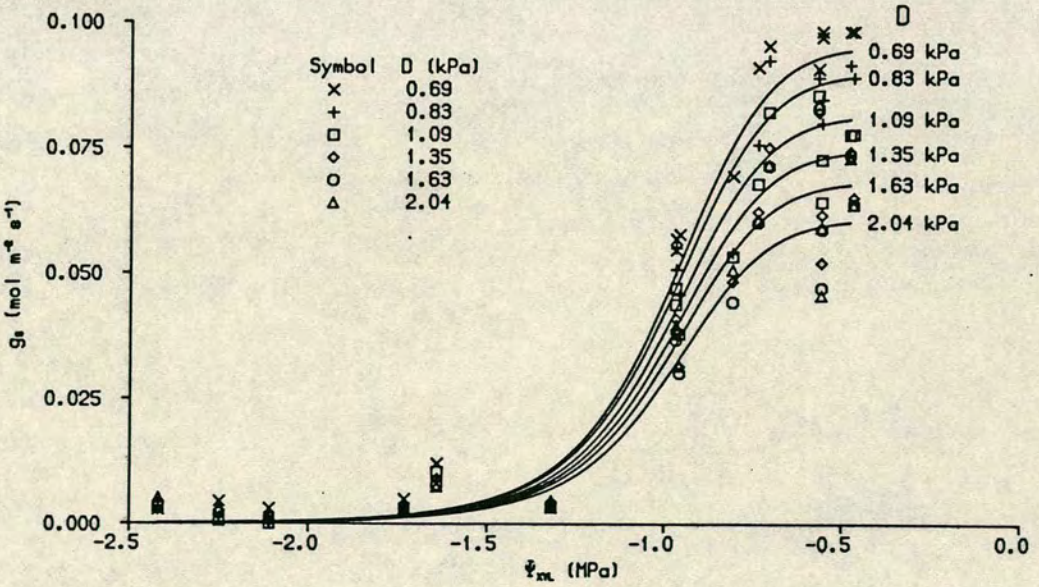

Figure 8.1b: g_s as a function of water potential for Sitka spruce at 6 levels of D. The data points are for 4 replicates. See the text for a description of the fitted curves.

E is plotted as a function of water potential in figures 8.2a+b, for Scots pine and Sitka spruce, respectively.

Using this model, graphs of g_s and E (figures 8.3a+b and 8.4a+b) were also generated to show these variables as functions of D, for five different levels of water potential. Unfortunately, because of the problem of reproducing levels of water potential, plotting the original data on these graphs is difficult. The likely goodness of fit can be judged from figures 8.1 and 8.2.

Defining a model, with some physiological meaning, for the response of A to D and to water potential is a much harder proposition as A may decline partly as a result of stomatal limitation and partly as a result of the direct effects of water stress on the biochemical processes of photosynthesis (Jones, 1973a). To determine the exact degree of limitation attributable to the stomata one must be able to define the A/C_i relationship at each level of stress (see Chapter 9 and Farquhar & Sharkey, 1982). This relationship was not established in these experiments and consequently critical analysis of stomatal limitation is not possible. However, it is possible to make some estimate of the likelihood of any direct effects of water potential on A by studying the A *versus* g_s relationship. If there was a marked decline in the biochemical capability for photosynthesis, one would expect a smaller value of A at the same value of g_s (see Schulze & Kuppers, 1979).

The data for A as a function of g_s, for all replicates at all levels of water potential, are shown in figures 8.5a+b, for Scots pine and Sitka spruce respectively. The measurements made during one day, at the same water potential, are joined by solid lines. In addition, the dashed line represents a curve fitted to all the points for A as a function of g_s, using a rectangula hyperbola of the form:

$$A = \frac{(C_a - \Gamma)g_s g_m}{(g_s + 1.6g_m)} \qquad 8.4$$

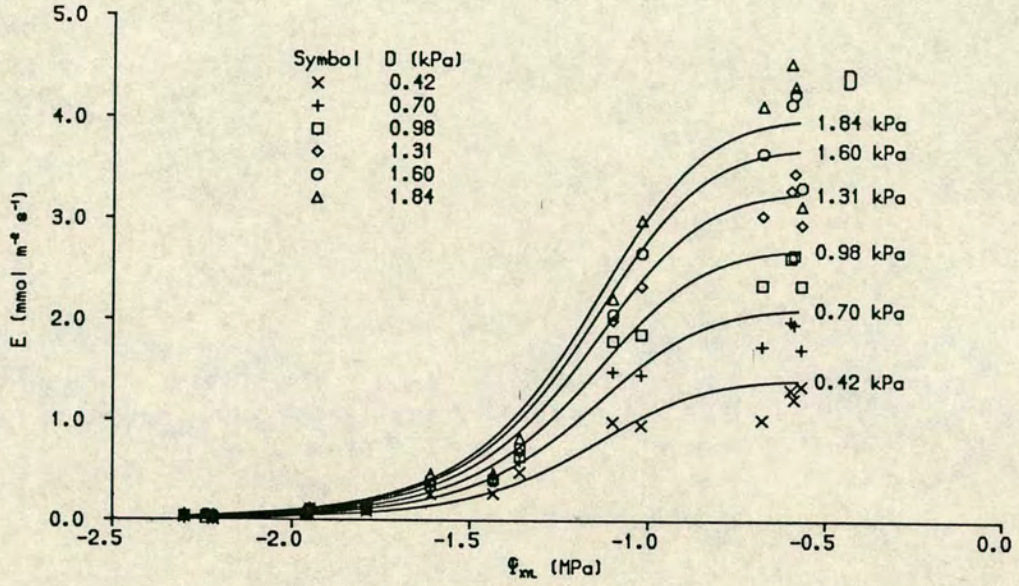

Figure 8.2a: E as a function of water potential for Scots pine at 6 levels of D. The data points are for 3 replicates. See the text for a description of the fitted curves.

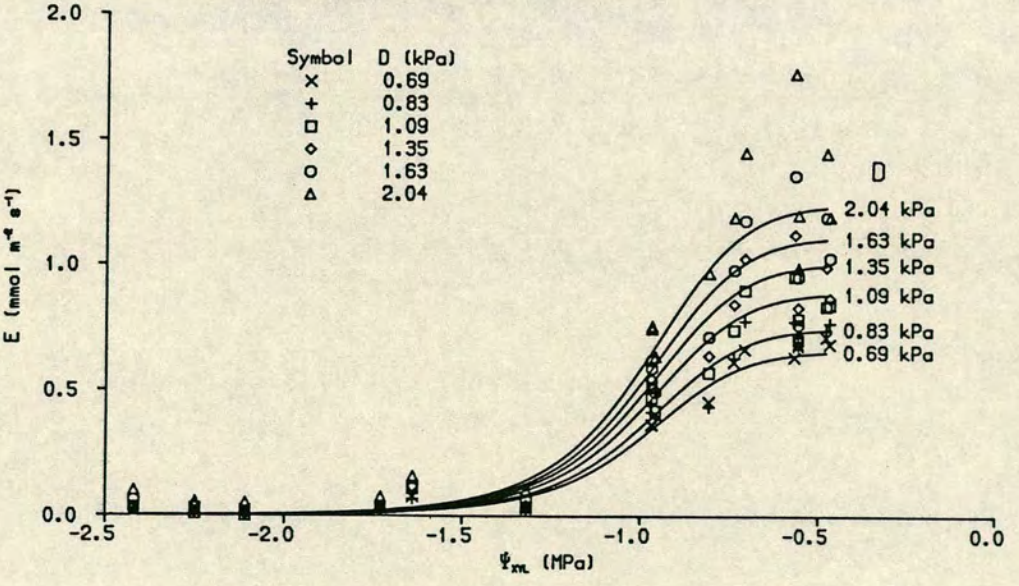

Figure 8.2b: E as a function of water potential for Sitka spruce at 6 levels of D. The data points are for 4 replicates. See the text for a description of the fitted curves.

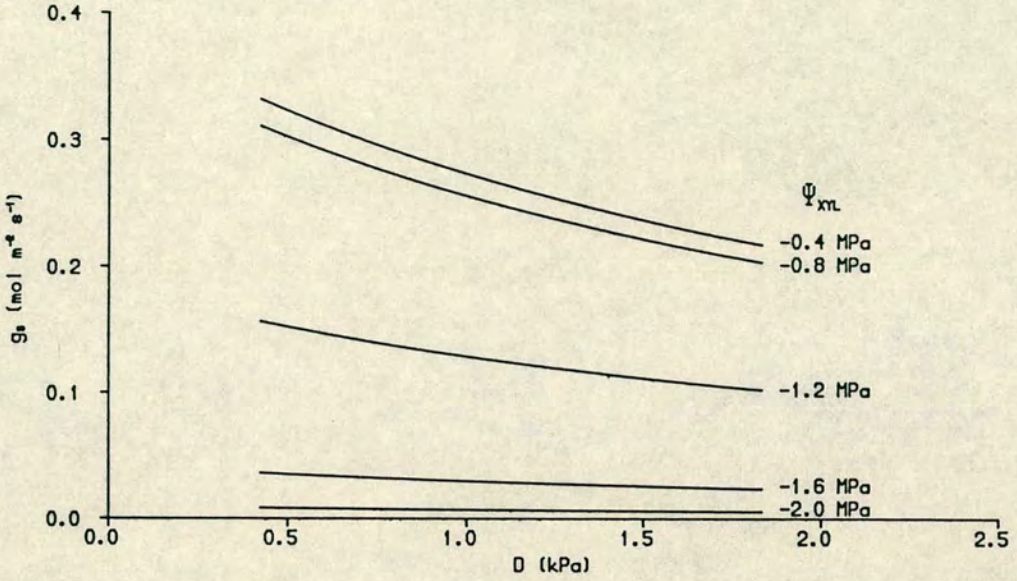

Figure 8.3a: Predicted g_s as a function of D for Scots pine at 5 levels of water potential. See the text for a description of the fitted curves.

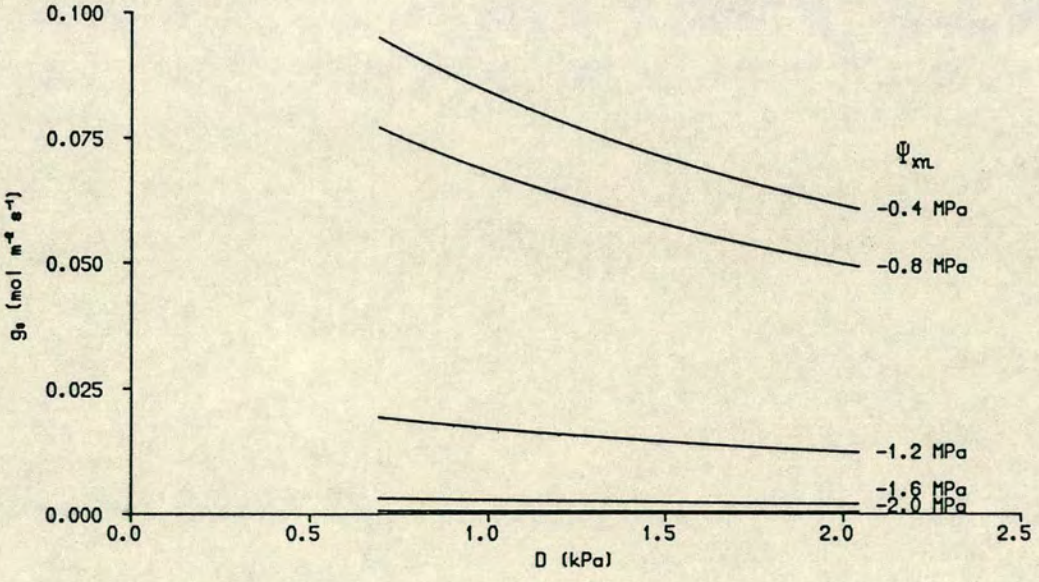

Figure 8.3b: Predicted g_s as a function of D for Sitka spruce at 5 levels of water potential. See the text for a description of the fitted curves.

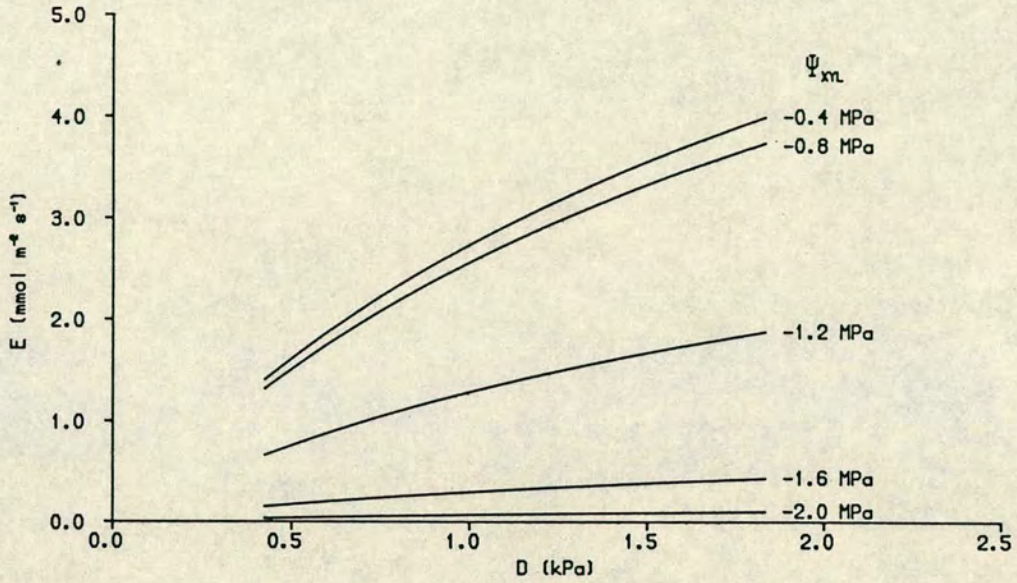

Figure 8.4a: Predicted E as a function of D for Scots pine at 5 levels of water potential. See the text for a description of the fitted curves.

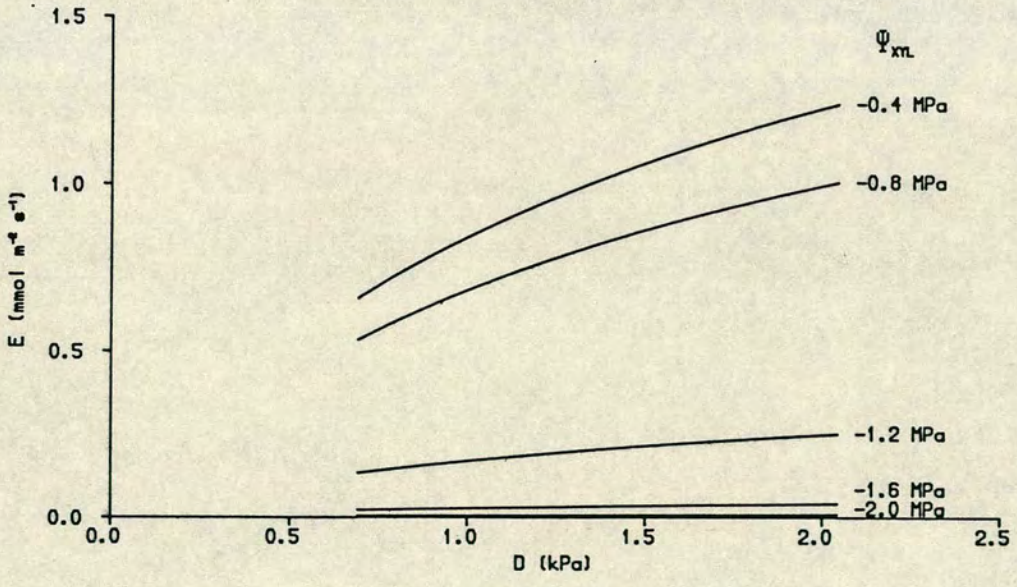

Figure 8.4b: Predicted E as a function of D for Sitka spruce at 5 levels of water potential. See the text for a description of the fitted curves.

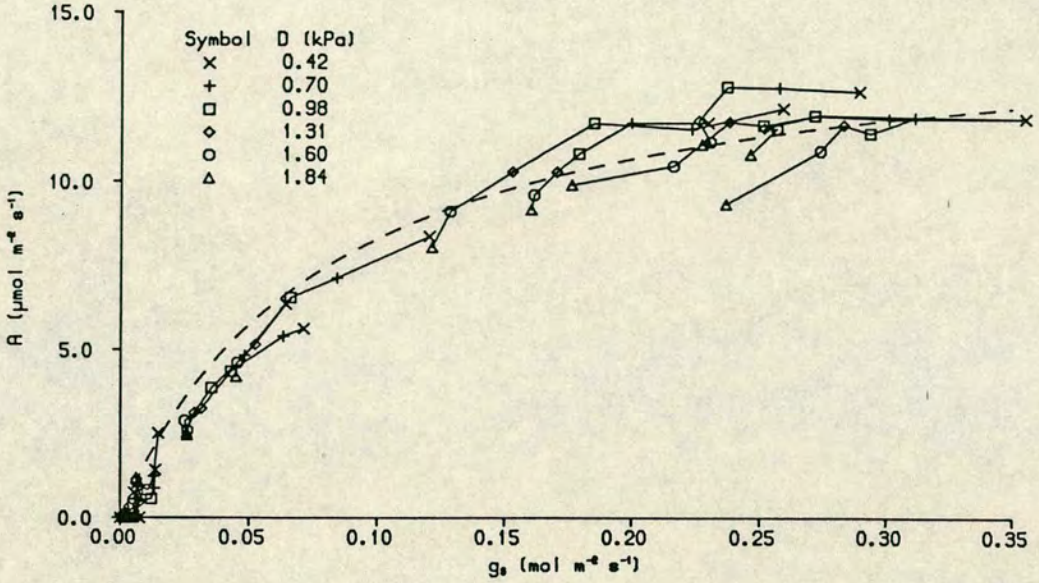

Figure 8.5a: A as a function of g_s for Scots pine, for all data. The data points for each day's experiment are joined by straight lines. See the text for a description of the dashed, fitted curve.

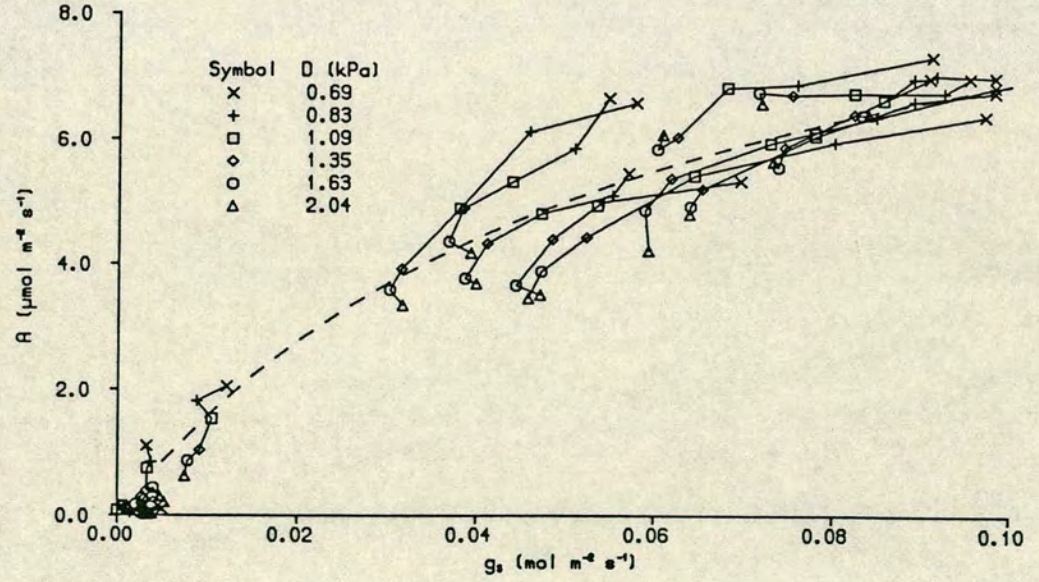

Figure 8.5b: A as a function of g_s for Sitka spruce, for all data. The data points for each day's experiment are joined by straight lines. See the text for a description of the dashed, fitted curve.

This relationship is derived by introducing the concept of mesophyll conductance (g_m). This parameter is the initial slope of the A/C_i relationship (Jarvis, 1971). To derive equation 8.4 it is necessary to assume that the plants 'operate' only on the linear region of the A/C_i curve, i.e.

$$A = g_m (C_i - \Gamma) \qquad 8.5$$

It is also necessary to assume that g_a is large enough so that it can be ignored. Using the approximation to equation 2.12, i.e.

$$A = g_{sc} (C_a - C_i) \qquad 8.6$$

By solving equation 8.5 for C_i, then substituting the result into equation 8.6 and converting g_{sc} to g_s (see Chapter 2), equation 8.4 can be found.

Beadle *et al* (1981) used an equation identical to 8.4 for assessing stomatal limitation of A. Watts & Neilson (1978) used an equation similar to 8.4 to calculate g_m, viz.

$$A_g = \frac{C_a g_{sc} g_m}{(g_{sc} + g_m)} \qquad 8.7$$

The differences between the equations lies in their measurement of gross, rather than net, A in their experiments.

If data for different water potentials all lie on the same A/g_s curve, fitted by equation 8.4, then, if the plants operate on the linear region of the A/C_i curve, it is likely that g_m is constant and independent of water potential, i.e. there is no direct effect of water potential on the carboxylation process (see Farquhar & Sharkey, 1982).

For these data it was felt inappropriate to include C_a or Γ as floating parameters as, in the case of C_a, it was known to be constant to within *ca* ±5%. Beadle *et al* (1981), studying Sitka spruce, found Γ not to change significantly from 40 μmol mol^{-1} over the range of water potential of

-0.5 to -2.0 MPa. Thus, in fitting the model to the A/g_s data, a fixed, round value of 300 μmol mol^{-1} was assumed for $(C_a - \Gamma)$, leaving g_m as the only parameter to be found. The values of g_m, derived from fitting equation 8.4 are given in table 8.3, for the two species.

Table 8.3 g_m derived from fitting a rectangular hyperbola (equation 8.4) to all of the A *versus* g_s data. The asymptotic standard deviation is given in brackets. The units for g_m are mol m^{-2} s^{-1}.

Species	Derived value of g_m	No. of measurements
Scots pine	0.0498 (±0.0007)	90
Sitka spruce	0.0362 (±0.0009)	102

As the data do not appear to show any marked trends away from the common fitted line, as shown in figures 8.5a+b (see Discussion below), the relationships derived using equation 8.4 could be used to predict A, by substituting in values of g_s predicted by equation 8.2, for different levels of D and water potential. This assumes that A is a function of g_s alone and that there is no direct effect of water potential on A.

The predicted curves of A are plotted as a function of water potential in figures 8.6a+b, for Scots pine and Sitka spruce, respectively. Six levels of D, each corresponding to the mean value of D imposed in steps during the experiment are shown. The individual data points are also plotted on these graphs to show the fit of the data.

The model was also used to generate curves for the relationships between A and D for five different levels of water stress (figures 8.7a+b).

As the model of A is based on the rather thin evidence of the A/g_s graphs, it was felt unwise to use this model to extrapolate further to predict E/A. Instead, E/A calculated from the raw data are plotted in figures 8.8a+b to show the trends in E/A. As the accuracy of the determination of E/A is dependent on the accuracy of measurement of

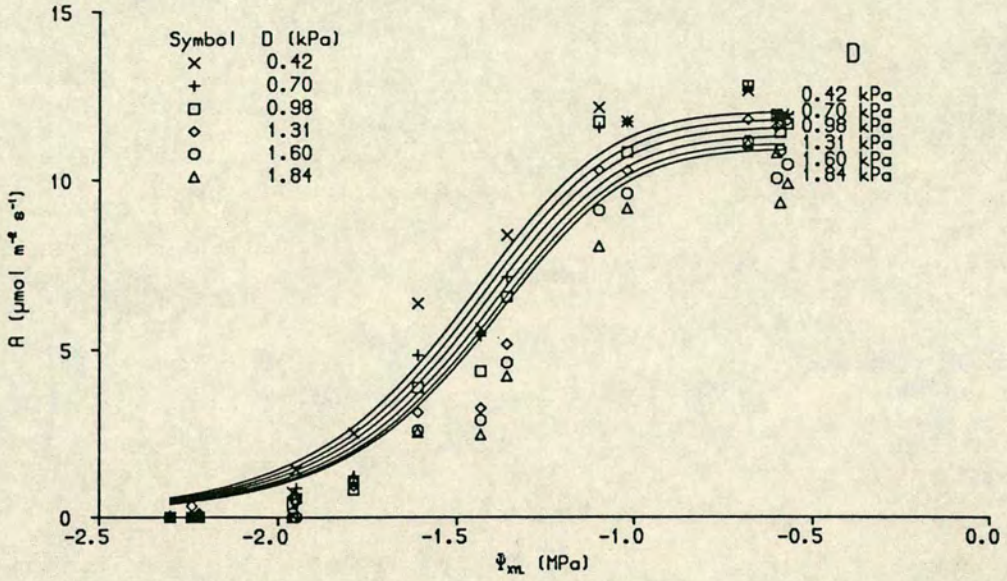

Figure 8.6a: A as a function of water potential for Scots pine at 6 levels of D. The data points are for 3 replicates. See the text for a description of the fitted curves.

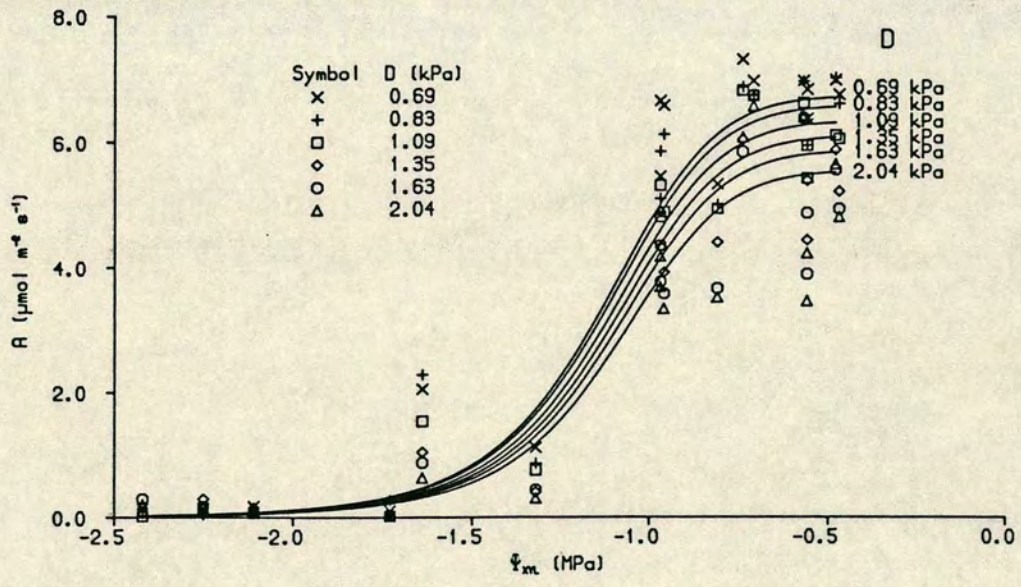

Figure 8.6b: A as a function of water potential for Sitka spruce at 6 levels of D. The data points are for 4 replicates. See the text for a description of the fitted curves.

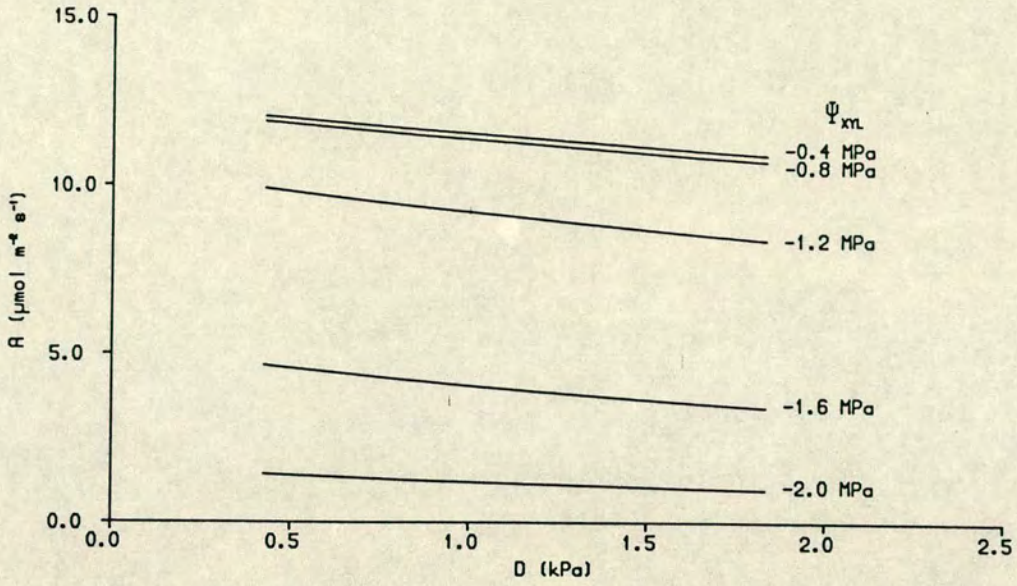

Figure 8.7a: Predicted A as a function of D for Scots pine at 5 levels of water potential. See the text for a description of the fitted curves.

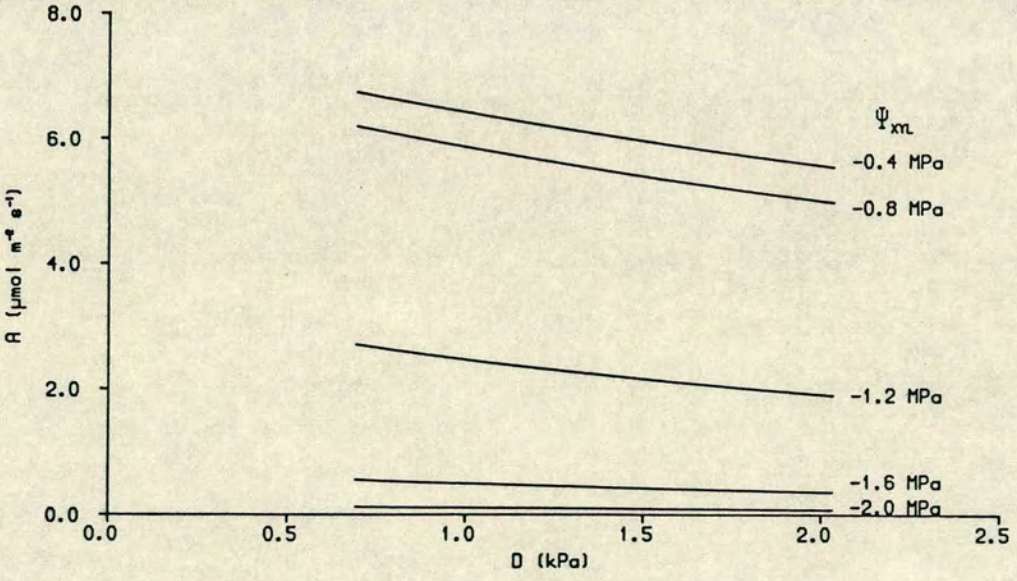

Figure 8.7b: Predicted A as a function of D for Sitka spruce at 5 levels of water potential. See the text for a description of the fitted curves.

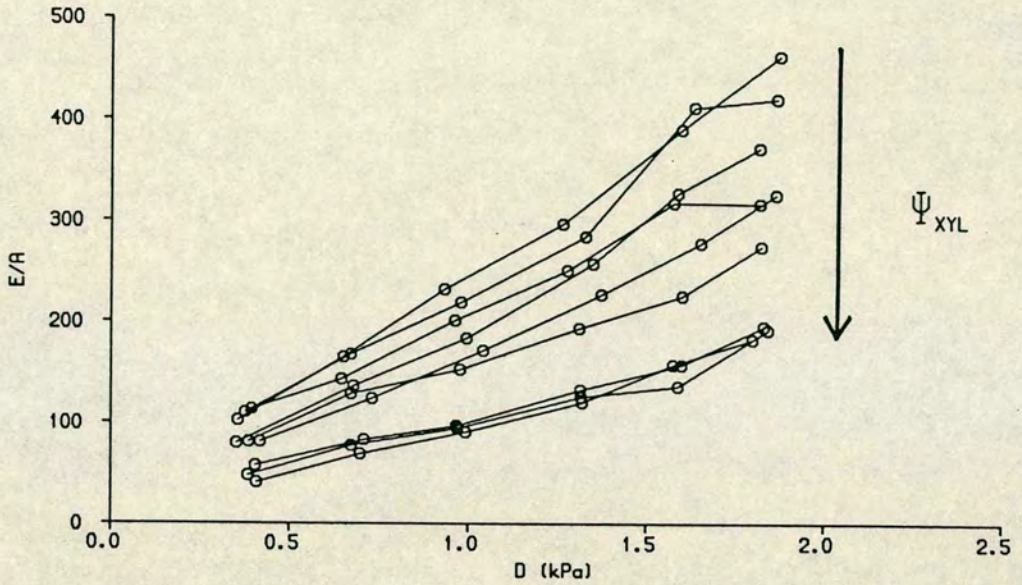

Figure 8.8a: E/A as a function of D for Scots pine. The data points for selected (see text) daily experiments are joined by straight lines. The arrow indicates a trend for the water potential to get more negative.

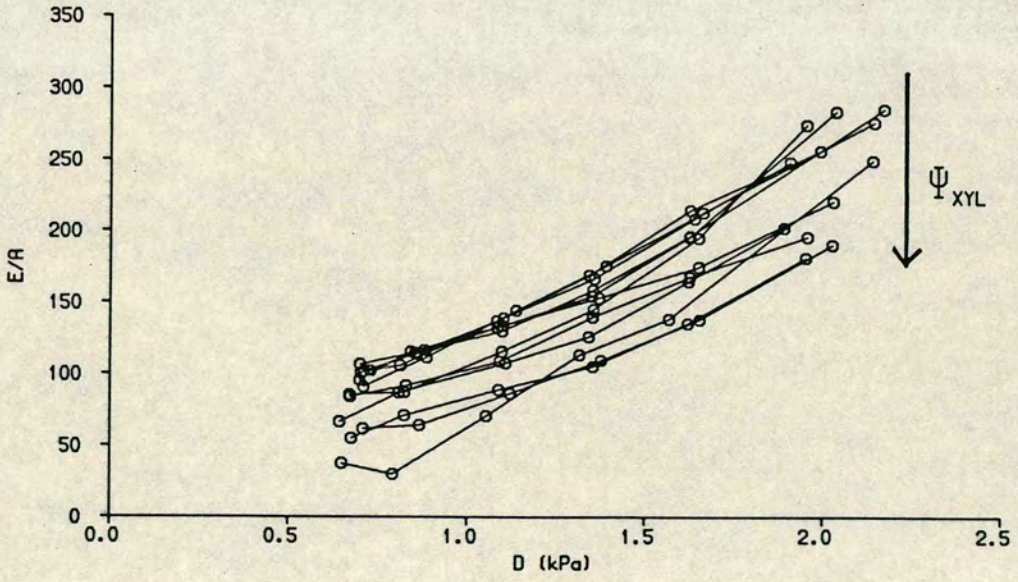

Figure 8.8b: E/A as a function of D for Sitka spruce. The data points for selected (see text) daily experiments are joined by straight lines. The arrow indicates a trend for the water potential to get more negative.

both E and A, when E and A were very small some unrealistic estimates of E/A were found. Therefore data are only presented for the individual experiments in which over 50% of the measurements of E and A were greater than 10% of the maximum value of E or A measured for all of the data.

The measurements of xylem water potential were very similar to those described in Chapter 3, i.e. there was only a slight decline in water potential over the course of a day: the average decline in water potential was -0.083 (s.e.±0.016) MPa for all of the Scots pine data and -0.061 (s.e.±0.023) MPa for all of the Sitka spruce data. There was a slight trend for smaller declines in water potentials when the water potential was low, probably because of the much reduced E. These small changes in water potential were considered unlikely to have any marked effect on the analysis of the results, for which the mean value of the morning and evening measurements were used in all cases.

The results of the experiments done after rewatering are not presented here as it was found that the prolonged period of stress at low water potentials had visibly damaged the plants. The tips of the needles of both species were yellowing and in some cases were brown because of tissue death. Analysis of such results, in terms of stomatal responses, is very difficult, as the reduced stomatal conductance found may be partly attributable to a reduction in effective leaf area.

8.5 Discussion

At high water potentials the stomata of the Scots pine shoots used in this experiment, showed broadly similar responses to D to those reported for the younger shoots in Chapter 3, i.e. a moderate response of g_s to D, amounting to *ca* 30% closure as D was increased from the 0.6 to 2.0 kPa.

In contrast the responses of the Sitka spruce seedlings differed markedly from those reported in Chapter 3. The maximum value of g_s was much smaller and the response to D was not as strong, being similar to that of Scots pine in proportional terms. The low values of conductance

resulted in increased errors of measurement, particularly at low water potentials. This resulted in an increase in the scatter in the data. The smaller absolute values of conductance could, in part, be a consequence of the forced, early bud break of these plants.

In both species the response of the stomata to water potential, at all levels of D, appeared to follow a sigmoid course, that could be called a threshold response. However, to justify this, more data at higher water potentials are required to show that no further increases in g_s would occur for higher potentials than those found in these experiments. The curves for Sitka spruce appear to show a sharp cut-off water potential, below which the stomata closed. However, this may be partly attributable to the lack of data points in the range of -1.0 to -1.5 MPa water potential, the curve fitting procedure being strongly weighted by the more numerous points at the extremes of water potential.

As discussed in Chapter 2, there are problems in quantifying the errors associated with the parameters derived from multivariate curve fitting. However, visual comparison of the data and the model shows that for both g_s and E the model describes the Scots pine data fairly well. For Sitka spruce there appears to be some slight underestimation of g_s, and therefore E, at the lower water potentials. This is also probably the result of poor distribution of the data with respect to water potential.

Parameter b in equation 8.2 is useful in determining objectively the degree to which water potential reduces g_s; b is analogous to the half-life of a radioactive isotope. It is possible to compare the values in table 8.2, with values estimated from other workers data. The value of -1.2 MPa for Scots pine is very similar to a value of *ca* -1.1 MPa for similar potted Scots pine seedlings obtained by Ng (1978). However Ng (1978) reported that Whitehead, and also Roberts, had communicated to him that they had independently measured lower thresholds for stomatal closure for Scots pine trees in the field. The value of -0.98 MPa for Sitka spruce, although not dissimilar to that for Scots pine, is markedly higher than the values that can be found in the literature. The value of b for potted Sitka spruce for the work of Watts & Neilson (1978) is *ca* -1.7 MPa and for shoots taken from mature trees is *ca* -2.2 MPa (Beadle

et al; 1979). A full discussion of the threshold for stomatal closure is given in Chapter 9.

The model chosen to fit the data assumes no synergistic interaction between D and water potential. However, the fit of the data to the model for g_s, for both species, is visibly good enough for it to be said that, in absolute terms, there is less response to D as water potential declines below *ca* -0.7 MPa. Thus these results are unlike the laboratory findings of Schulze & Kuppers (1979) for *C. avellena*, Osonubi & Davies (1980) for *B. pendula*, Johnson & Ferrell (1983) for Douglas-fir or Ng (1978) for Scots pine, all of whom showed that the sensitivity of g_s to D either increased at intermediate water potentials or remained constant (see above). To test conclusively whether the plants, used in these experiments, exhibited greater sensitivity to D at intermediate water potentials, it is desirable to have more data for water potentials in the range of -0.7 to -1.4 MPa and also for potentials > 0.4 MPa. The latter being required to see if the response to D might decrease at very high potentials.

The fit of the model to the data suggests that the assumption that the responses of g_s to D and water potential do not interact is adequate. Thus the response of g_s to D follows the proposal of Morison & Gifford (1983), as discussed in Chapter 3, i.e. dg_s/dD is proportional to the absolute magnitude of g_s. In these experiments the absolute magnitude of g_s for the same value of D was determined by the level of water stress.

The plots of A *versus* g_s for both species showed that overall the data lie on a common curve. The scatter of the data for Sitka spruce appears to lie around the line for the whole range of g_s. For Scots pine, although the curve generally fits the data, there is a distinct trend for A to be overestimated at the intermediate values of g_s and underestimated at higher values of g_s. There are several explanations for this discrepancy.

i) That g_m decreases at lower water potentials.

ii) That Γ changes significantly with water potential.

iii) That, for the larger values of g_s, the plant is operating on the non-linear region of the A/C_i curve and in addition the asymptote of the A/C_i relationship may have changed with water potential.

Any one, or any combination of the above may explain the fit of the curve, but unfortunately it is impossible to distinquish which, from the A/g_s data alone.

For each day's measurements of the change in g_s in response to D, the lines joining the points appear to lie across the commom A/g_s curve, with points at higher D being below the line. If the trends of the joined points curves are extrapolated to the x-axis they generally appear to cut the axis with g_s significantly above zero. This is highly unlikely and can only really be interpreted as being caused by changes in the A/C_i relationship as D was increased during the course of each daily experiment. This is a similar phenomenon to the trends in A discussed in Chapters 4 and 5; see Chapter 9 for further discussion.

This method of trying to assess the effect of water potential on A is not very sensitive to the effects of very low water potentials on A, as it is impossible to determine precisely the shape of the A/g_s curve at low g_s because of errors in measurement. It is clear from the signs of needle yellowing that A was reduced directly at extremely low water potentials, but when g_s is so small it is impossible to study these effects using conventional 'open' gas exchange techniques.

Despite these problems for the A/g_s analysis, the fitted values of g_m (table 8.3) fall into the range of those reported by previous workers for conifers (Jarvis & Leverenz, 1981). The value for Sitka spruce is almost identical to the value of 0.037 mol m^{-2} s^{-1} found by Watts & Neilson (1978) for Sitka spruce, using a similar technique of analysis (see above). However, both of these values are lower than the value of 0.05 mol m^{-2} s^{-1} found by Beadle *et al* (1981) for shoots of Sitka spruce for this range of water potentials. The value for Scots pine is slightly lower than found by Linder & Troeng (1980) for fertilized trees in the field

(0.068 mol m^{-2} s^{-1}), but considerably higher than their unfertilized trees (0.036 mol m^{-2} s^{-1}).

The results for Sitka spruce of Beadle *et al* (1981) support the hypothesis that g_m is probably constant over the range of potentials studied here, as they found no effect of water potential on g_m for potentials above -2.0 MPa. Their data were, however, for shoots taken from the field and, as stated above, the stomatal response to water potential they found was very different from that found for these plants.

The prediction of A as a function of D and water potential (figures 8.6a+b) appears, from the distribution of the data points not to be as successful in comparison to the fit of equation 8.4 to g_s (figures 8.1a+b). This is partly a result of the curves being an extrapolation of the g_s model, which is not totally adequate. Thus some poorness of fit can be attributed to deviation in the fit of the g_s model. Much of the remaining scatter is because the individual daily measurements of the response to D lie across the common A/g_s curve (see above). Thus for both species the model represents an underestimate of the range of response of A to D, although the trends with water potential appear to be adequately described. It is interesting though that the predicted function of A *versus* D (figures 8.7a+b) results in a virtually linear relationship, as found previously (Chapter 3).

The trends for E/A as a function of D (figures 8.8a+b) show that, as has been found previously, E/A increased as D increased, for both species. The arrows in the figures indicate the trend for the water potential to decrease as E/A declines, i.e. the plants used water more efficiently with respect to CO_2 fixed, at lower water potentials. There is some evidence for a similar decline in E/A for *B. pendula* in the work of Osunubi & Davies (1980), although there was no similar trend for *G. arborea* in the same study. A significant increase in E/A at lower water potentials is clearly of important ecological significance as it means that the reduction in g_s to reduce E will cause less of a decline in A (Jones & Mansfield, 1972).

To summarise, for both Scots pine and Sitka spruce the response of g_s to D appears to be dependent on the absolute magnitude of g_s and independent of water potential, the absolute magnitude of g_s being determined by water potential. For Sitka spruce there is good evidence that there was no direct effect of water potential on A, for Scots pine the evidence is not so good. However, for critical analysis of the effect of stomatal closure on A, in response to D and water potential, the A/C_i relationship should be determined at each level of water potential. This was subsequently done and is described in the following chapter where more detailed discussion of stomatal limitation and E/A is given.

CHAPTER 9

STOMATAL LIMITATION OF PHOTOSYNTHESIS IN RESPONSE TO CHANGES IN LEAF WATER POTENTIAL AND LEAF-TO-AIR WATER VAPOUR PRESSURE DIFFERENCE

9.1 Introduction

In the previous chapter it was shown that stomatal closure, in response to D and water potential, can result in significant limitation of A. To understand the exact role of the stomata in the reduction of A it is necessary to determine if there are any direct effects of changes in leaf water potential on A. To do this precisely it is necessary to measure the A/C_i response curve, at each level of stress (Jones, 1973a). One can then estimate the limitation of A by the stomata (Farquhar & Sharkey, 1982; Jones & Fanjul, 1983).

From the analysis of the A/g_s data in the previous chapter, it was shown that there was a possibility of stomatal closure with little or no direct effects of stress on A. This possiblity is particularly interesting in the light of claims by Wong *et al* (1979) that g_s is highly correlated to photosynthetic capacity. The data in Chapter 8 would imply a very weak link between A and g_s. It was, therefore, decided to repeat the drying-cycle experiment with the addition of determining the A/C_i response as the stress developed.

9.2 Plant material

(1+2)-year-old seedlings of Queen Charlotte Islands provenance Sitka spruce were used for this experiment. These plants broke bud at the normal time of year, i.e. early June, outside and at the start of the experiment the shoots were five months old.

In an attempt to slow down the rate at which the water potentials dropped at the intermediate levels of stress, these plants were repotted approximately 12 weeks prior to the start of the experiment, into

300 mm diameter pots using John Innes No. 2 compost. It was hoped that the higher loam content of this soil would result in a slower decline in soil water potential. However, to prevent damage to the fine root system the original peat-based compost was not removed from the 'root ball'.

For logistical reasons these plants were preconditioned in a Fisons growth cabinet (see Chapter 2). The daylength and temperature conditions were identical to those used previously. The main difference in the conditions in this cabinet was the photon flux density at the shoot level, of *ca* 750 μmol m^{-2} s^{-1}. This is almost twice that in the growth rooms used previously.

9.3 Experimental details

The drying cycle was applied in an identical way to that described in Chapter 8. After an initial measurement, watering was stopped and the response to D was measured every five days for each of four replicate plants. A total of four measurements were made on each replicate. The stomata were essentially closed for the last measurements. As in the experiments described in Chapter 8, there were still visible signs of damage to the needles, e.g. yellowing of the needle tips, despite rewatering after the last measurement. Therefore, no attempt was made to make further measurements.

In addition to measurement of the responses of g_s and A to D, the A/C_i response was determined at the start of each experiment. The exact procedure was as follows.

After the lights were switched on, D was kept at the overnight level of 1.0 kPa. These conditions were held for a period of 1 hour to allow the stomata to achieve a reasonable degree of opening. Using the gas-mixing pumps, the CO_2 mole fraction entering the chamber (C_e) was then varied in steps to give mole fractions of 1000, 500, 400, 300, 200, 100 μmol mol^{-1}.

It was found that it took *ca* 20 min. for the CO_2 mole fraction to stabilise after changing the output of the gas-mixing pumps. A further 10 min. were required for calibration of the gas-analyser and to take a series of measurements. Thus each step of C_e and hence each point on the A/C_i curve took 30 min. to measure. To determine the complete A/C_i curve took three hours. As this length of time constitutes a substantial proportion of the day, no attempt was made to make corrections to C_i, either by selecting a different value of C_e or by adjusting the flow entering the chamber (F_e) (see Morison & Jarvis, 1983). Such adjustments would have prolonged the time taken to determine the A/C_i curve and therefore reduced the time left to measure the responses to D. However, this procedure, with predefined values of C_e, meant that the value of C_i, for which the measurement of A was taken, was variable as it depended on A and g_s, i.e. the independent variable was not truly independent. This led to problems in comparison of the resultant A/C_i curves (see below).

After the last point on the A/C_i curve had been recorded, the gas-mixing pumps were bypassed and the chamber supplied with air from outside with a CO_2 mole fraction of *ca* 340 μmol mol^{-1}. D was then set to the initial level for determination of the response of A and g_s to D, i.e. *ca* 0.6 kPa. This starting level of D was slightly higher than used previously (0.4 kPa) to avoid problems with condensation in the assimilation chamber, caused by failure of the laboratory air-conditioning system. The responses to D were then determined in an identical way to that described in Chapter 3.

As for the experiments in Chapter 8, xylem water potential was measured for a similar shoot at the start of the experiment and for a side shoot, of the shoot being studied, at the end of the experiment.

9.4 Techniques of data analysis and results

The response of g_s to D was analysed in an identical way to that described in Chapter 8, i.e. equation 8.2 was fitted to the g_s/D data for all replicates and water potentials, using the BMDP, PAR, non-linear,

least-squares package. The data were standardised with respect to the maximum level of A, measured at the highest water potential and highest CO_2 mole fraction for each of the A/C_i curves. Unlike the procedure followed previously, the same scaling for each replicate factor was also applied to the g_s and E data. This was done as independent standardisation of g_s could invalidate full interpretation of the A/C_i data. However, rescaling the g_s data, using the scaling factors derived for A, brought the values of g_s for the different replicates, at the reference condition for standardisation, to within ±5% of each other, i.e. much of the variation amongst the replicates could be attributed to morphological differences which affected g_s and A equally. The real mean of A for the four replicates, at the reference condition for standardisation, was 22.17 (s.e.±2.50) μmol m^{-2} s^{-1}.

The average of the morning and evening water potentials was used for all analyses (see below). The parameters derived from fitting equation 8.2, are given in table 9.1. The raw data and fitted curves are shown in figures 9.1 and 9.2 for g_s and E, respectively. Predicted values of g_s and E are also presented, as a function of D, for five levels of water potential, in figures 9.3 and 9.4, respectively.

Table 9.1 The parameters derived from a model (equation 8.2) fitted to the g_s, D and water potential data. Units for a are mmol m^{-2} s^{-1} kPa^{-1}, for E_m, mmol m^{-2} s^{-1}, for b, MPa and c is dimensionless. The asymptotic standard deviations of the parameters are given in brackets.

Parameters				No. of
a	E_m	b	c	measurements
3.847 (±0.132)	6.04 (±0.69)	-0.739 (±0.013)	4.317 (±0.408)	96

As can be seen from these data, there appears to be little effect of water potential on g_s above -0.7 MPa, but below this value the stomata close markedly. The A data were, therefore, initially divided around this level. Individual A *versus* C_i responses are plotted in fig. 9.5, with different symbols used for the two divisions of water potential. A/C_i

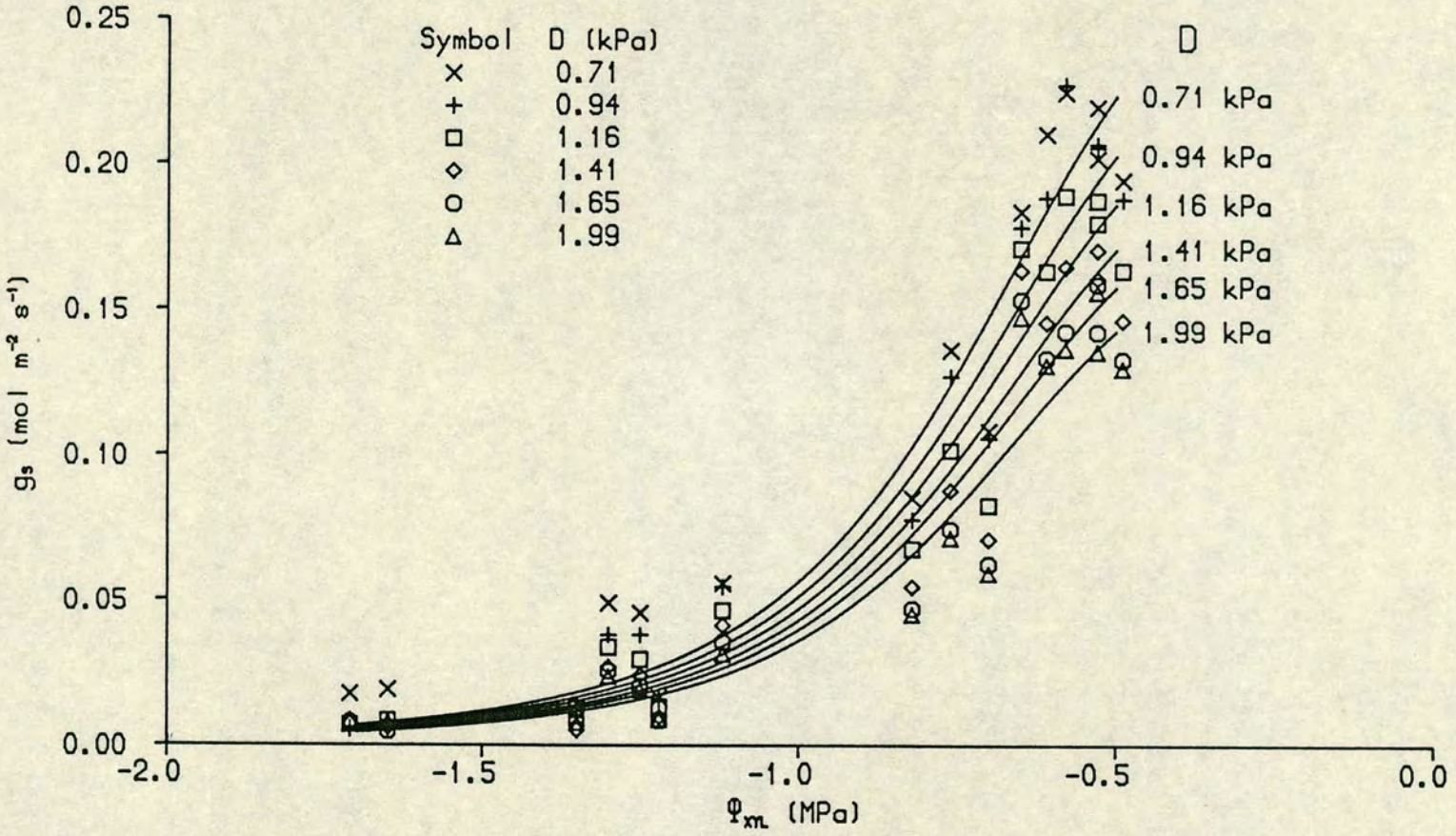

Figure 9.1: g_s as a function of water potential at 6 levels of D. The data points are pooled for 4 replicates. See the text for a description of the fitted curves.

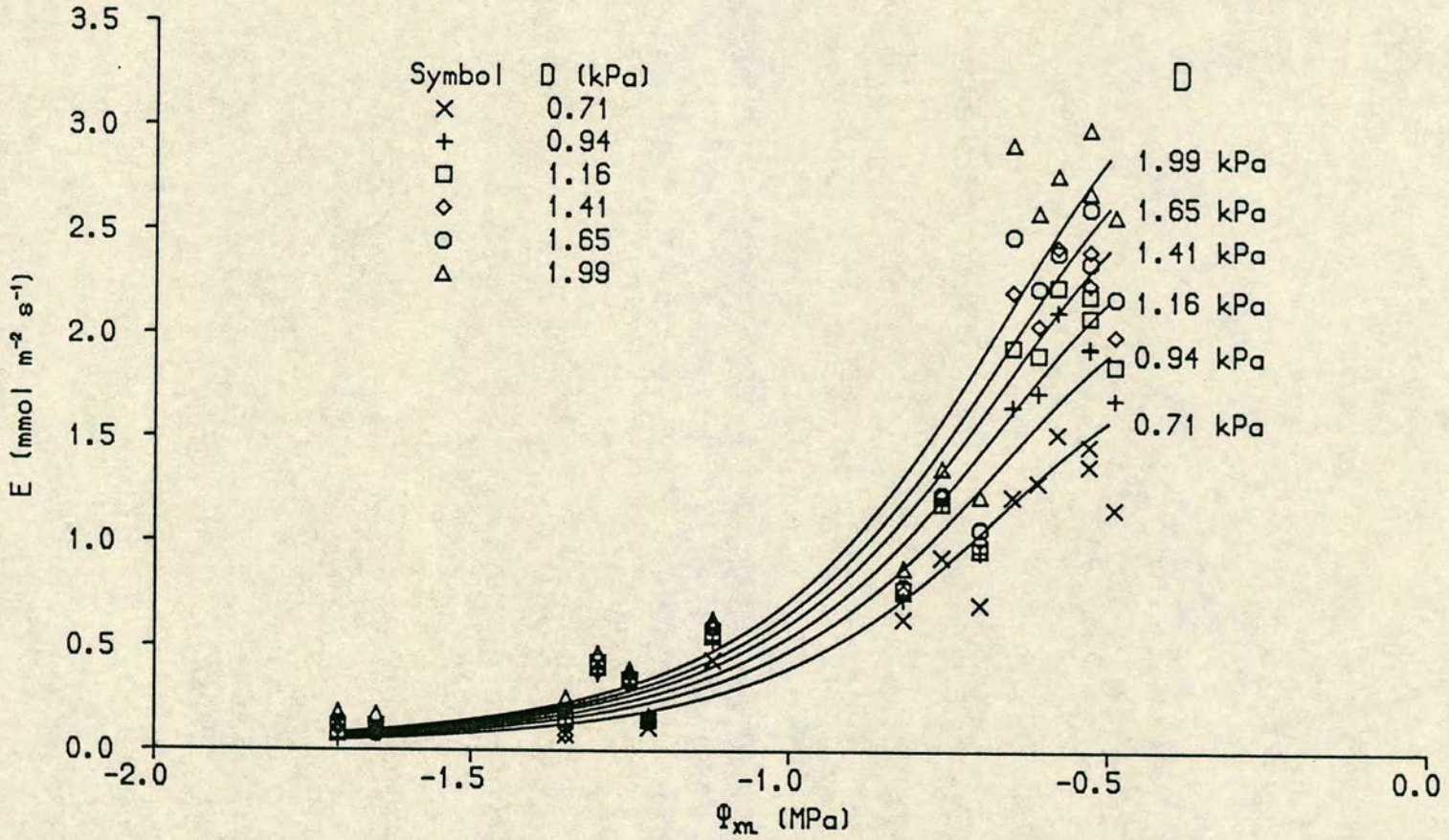

Figure 9.2: E as a function of water potential at 6 levels of D. The data points are pooled for 4 replicates. See the text for a description of the fitted curves.

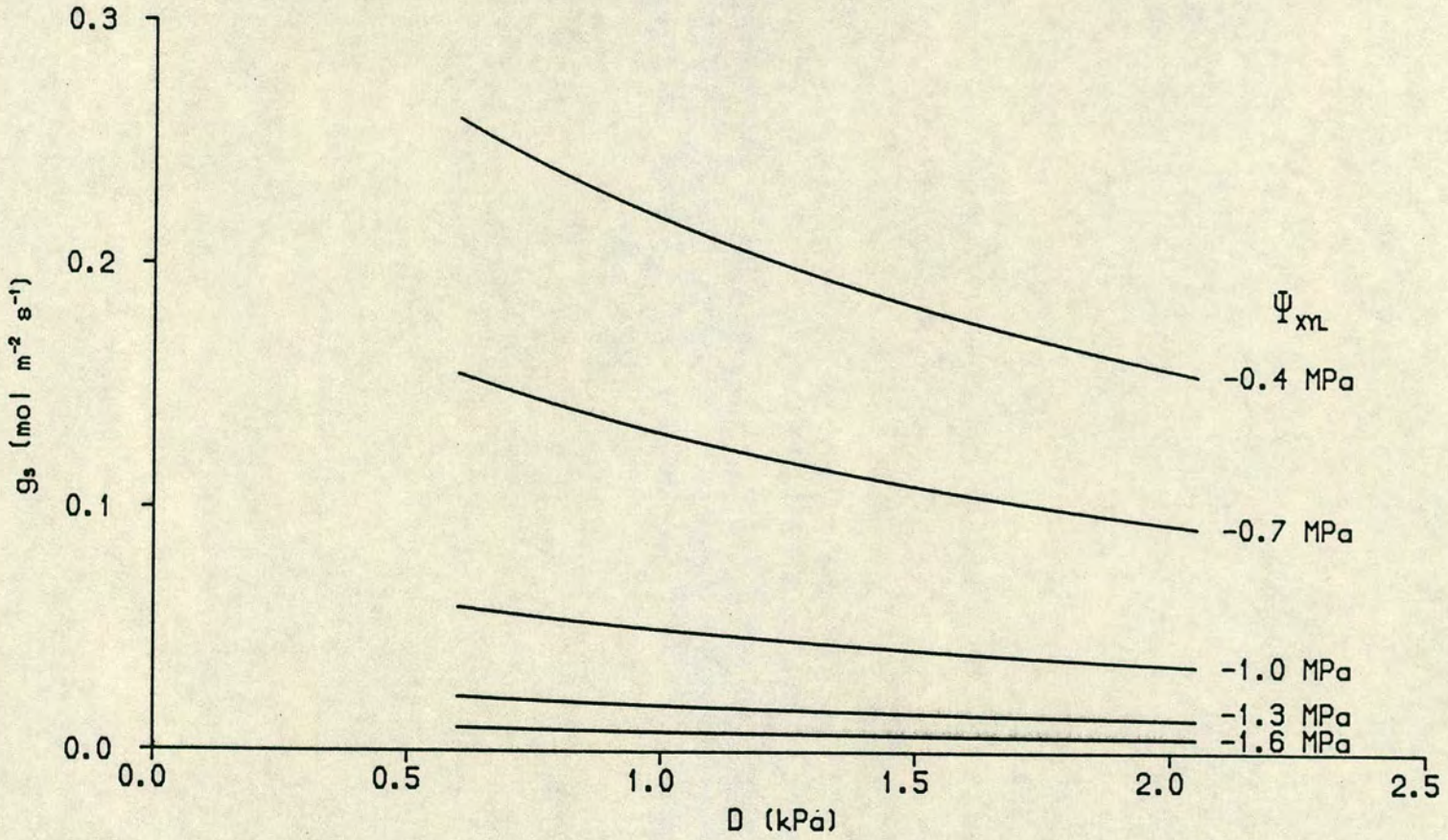

Figure 9.3: Predicted g_s as a function of D at 5 levels of water potential. See the text for a description of the fitted curves.

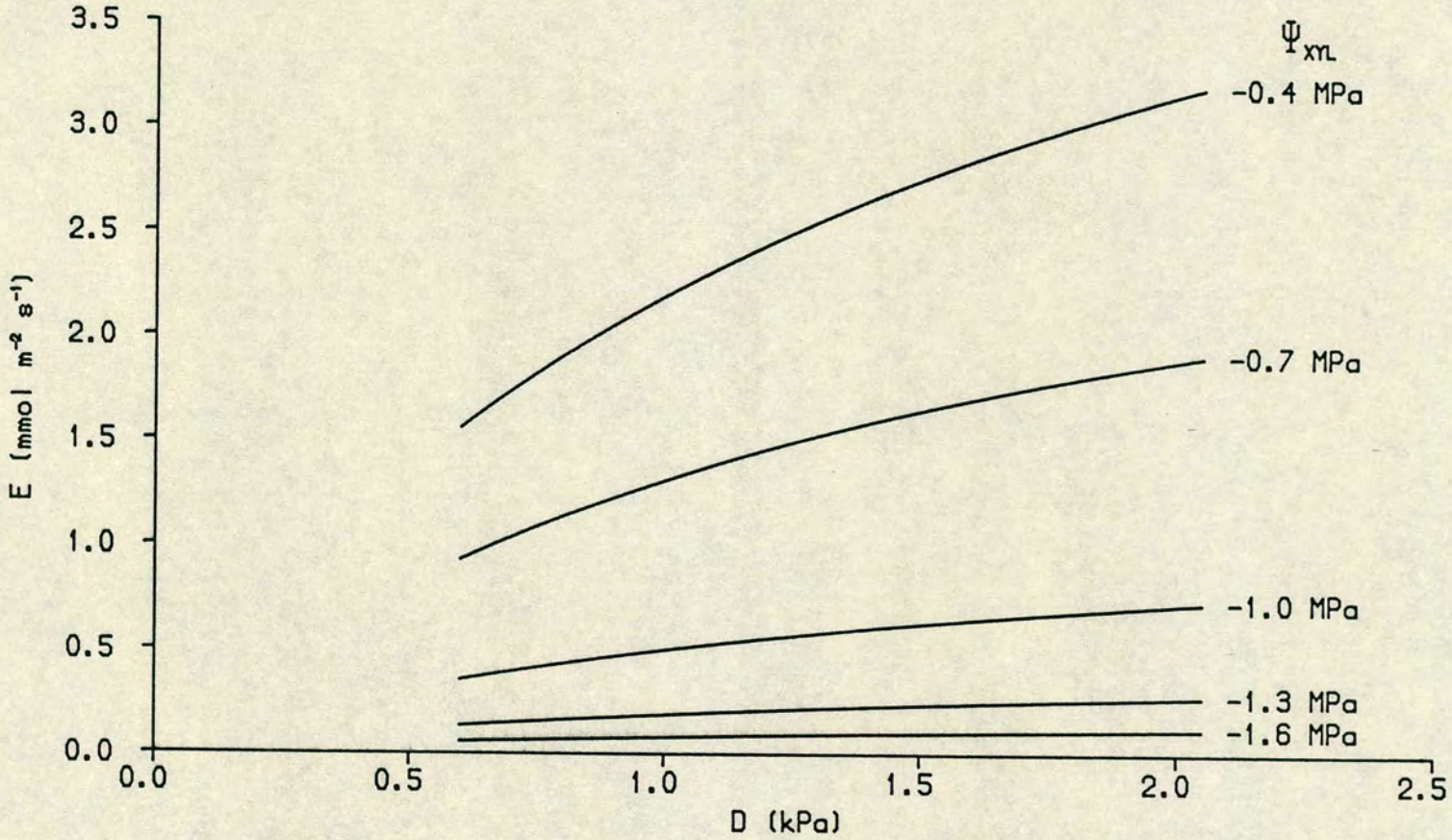

Figure 9.4: Predicted E as a function of D at 5 levels of water potential. See the text for a description of the fitted curves.

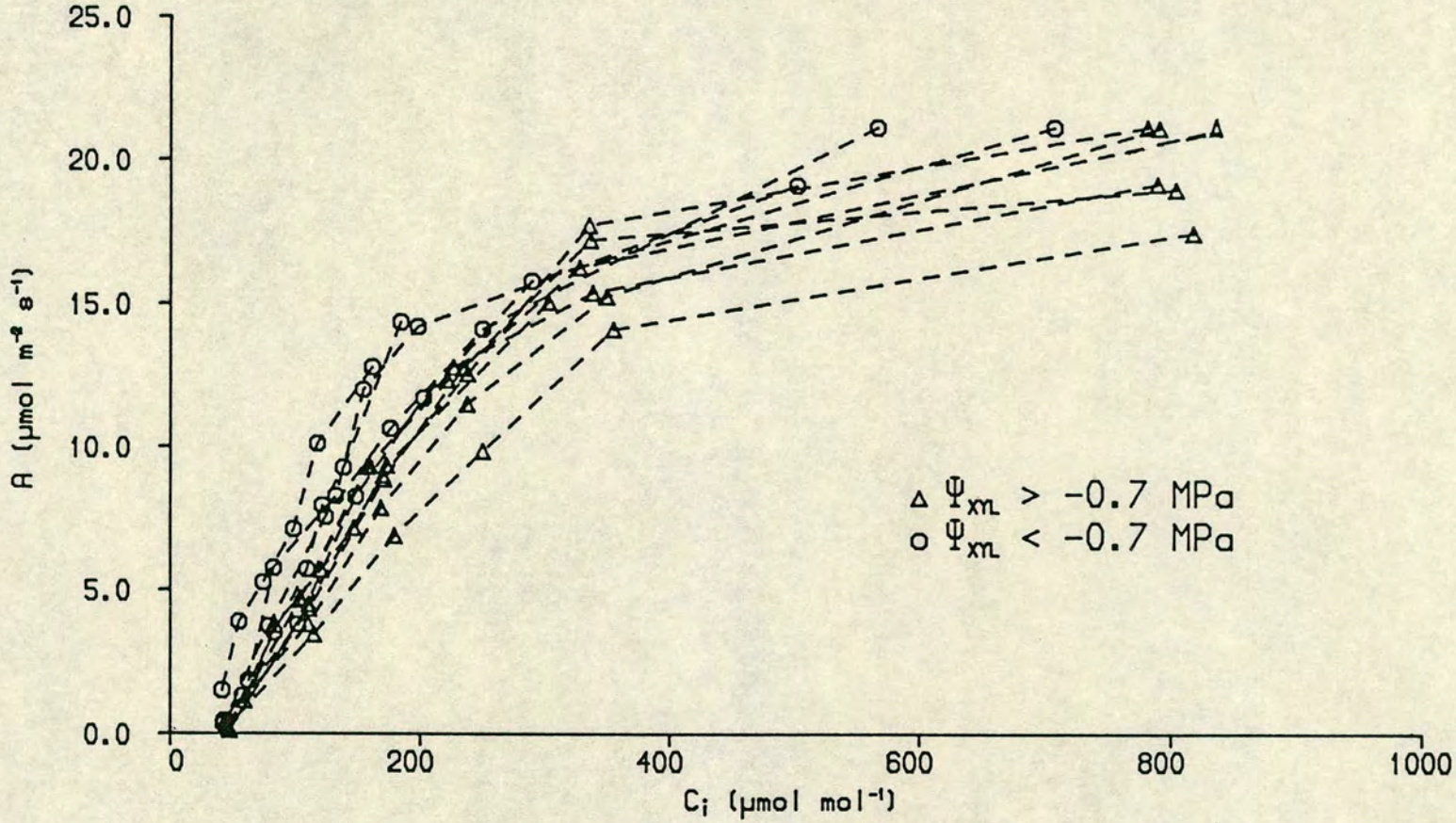

Figure 9.5: A as a function of C_i for selected data (see text). Responses measured on each day are joined by straight lines.

data for the first three sets of measurements made on each replicate are only presented and used in the analysis, as, for the fourth measurement, g_s and A were less than 5% of the maximum values (water potential < -1.2 MPa). Calculation of C_i was thus subject to large errors (see Chapter 2) and some negative values for C_i resulted.

All of the response 'curves' appear to have a similar form, with A increasing linearly initially and then curving off to reach an asymptote at higher levels of C_i. Visually there also appears to be some difference between the data for the two groups of water potential. To test whether there were any differences between the different classes of water potential, a model was required to describe the data.

Various models have been proposed to describe the A/C_i relationship. Several earlier models were based on a rectangular hyperbola, in an attempt to simulate the enzyme kinetics of the carboxylation reaction (Chartier & Prioul, 1976; Jones, 1983). However, such analyses do not describe A/C_i relationships which curve sharply to the asymptote, so non-rectangular hyperbolae have also been used (Jones & Slatyer, 1972). If one makes certain assumptions, one can assign various physiological parameters to these models.

Recently Farquhar *et al* (1980a) have developed a model based on the biochemical processes of photosynthesis. Although this model appears quite complex, the number of parameters for the model are not many more than for some of the previous alternatives. However, to fit the model, biochemical data are required together with estimation of the A *versus* Q relationship (von Caemmerer & Farquhar, 1981). Whilst it is possible to assume various estimates of the biochemical parameters, as done by Day & Parkinson (1982), no light response curves were measured for this experiment, so this model could not be fitted, even though conceptually it is possibly the best to date.

Rather than fitting a predetermined model based on resistance analogues, a general, non-rectangular hyperbola was fitted to the A/C_i data (Thornley, 1976). The x-variable used was $(C_i - \Gamma)$ giving an equation of the form:

$$A^2\theta - A(\alpha(C_i - \Gamma) + A_m) + \alpha(C_i - \Gamma)A_m = 0 \qquad 9.1$$

Where θ is the parameter that controls how sharply the curve bends to the asymptote (θ=0 gives a rectangular hyperbola; θ=1 gives two staight lines). α is the initial slope of the A/C_i curve and could possibly be interpreted as being equivalent to the mesophyll conductance (see below). A_m is the asymptotic value of A.

This equation can be solved for A, using the general formula for solving quadratic functions, as follows:

$$a = \theta$$

$$b = -(\alpha(C_i - \Gamma) + A_m)$$

$$c = \alpha(C_i - \Gamma)A_m$$

Thus $$A = \frac{-b - (b^2 - 4ac)^{0.5}}{2a} \qquad 9.2$$

The parameters derived by fitting this function to all the data, and also to the data grouped for above and below -0.7 MPa water potential, are shown in table 9.2. The curve fitted to all the data is also shown in fig. 9.6.

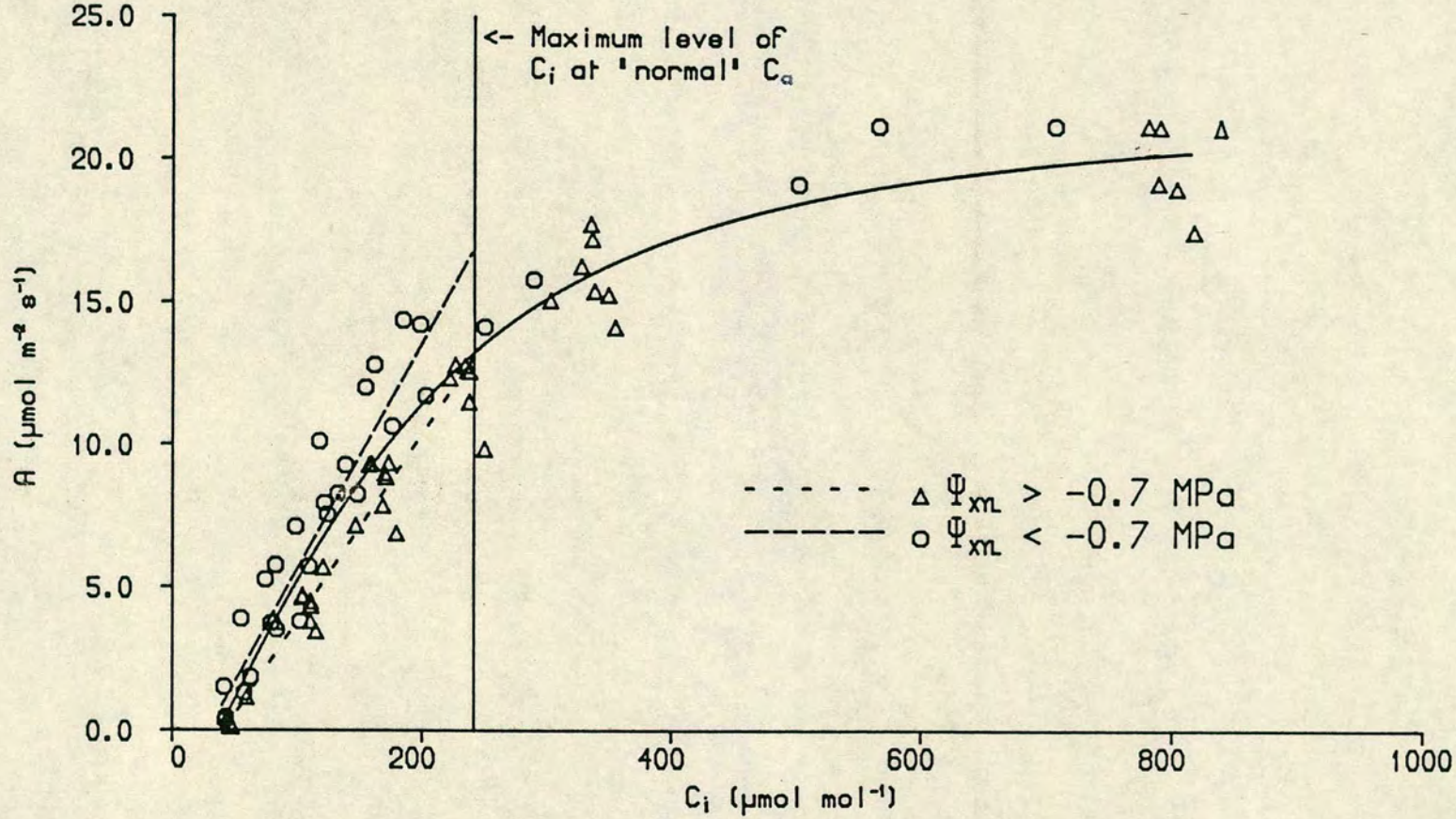

Figure 9.6: A as a function of C_i for selected data (see text).
The solid curve is a non-rectangular hyperbola fitted through all the points.
The dashed lines are fitted by linear regression on selected data (see text).

Table 9.2 The parameters derived from fitting a non-rectangular hyperbola to the A/C_i data. Units for α are mol m^{-2} s^{-1}, for A_m, µmol m^{-2} s^{-1}, for Γ, µmol mol^{-1} and θ is dimensionless. The asymptotic standard deviations of the parameters are given in brackets.

Data Set	Parameters θ	α	A_m	Γ	No. of measurements
All	0.7089 (±0.1802)	0.0916 (±0.0132)	22.61 (±1.60)	38.0 (±5.7)	72
ψ_{xyl} > -0.7 MPa	0.8888 (±0.0742)	0.0725 (±0.0080)	20.97 (±1.02)	44.0 (±6.7)	36
ψ_{xyl} < -0.7 MPa	0.6454 (±0.3668)	0.1045 (±0.0213)	24.44 (±3.50)	36.3 (±7.2)	36

Applying statistical tests to compare these parameters rigorously is very difficult (see Chapter 2). I therefore considered whether the section of the A/C_i curve in which the plant normally functions might be described by a simpler function, i.e. a straight line. This might allow the concept of mesophyll conductance to be applied to the data (Jarvis, 1971). To test this possiblity, the values of C_i, recorded whilst the responses of g_s and A to D were being measured, were scanned to find out what the maximum C_i was for these experiments. This was found to be 242 µmol mol^{-1} and is represented in fig. 9.6 by a solid line, parallel to the y-axis. Visually this mole fraction level appears to be below the point at which the A/C_i relationship curves over markedly.

Thus I felt justified in fitting a straight line of the form:

$$A = g_m C_i + R_l \qquad 9.3$$

The parameters for fitting this line, for values of C_i below 242 µmol mol^{-1}, to all of the A/C_i response 'curves' and to the data split above and below -0,7 MPa water potential (using Presto, see Appendix 4) are given in table 9.3. The straight lines for the latter two data sets are also shown in fig. 9.6. From these lines it was possible to calculate the CO_2 compensation mole fraction (Γ) as $-R_l/g_m$, these values are also given

in table 9.3. Equation 9.3 can thus be rewritten in an identical form to equation 8.4.

Table 9.3 The parameters derived from a linear regression of A as a function of C_i. Standard errors are given in brackets. The maximum C_i included in the analysis was 242 μmol mol^{-1}. The units for the slope (g_m) are mol m^{-2} s^{-1}, for the intercept (R_l), μmol m^{-2} s^{-1}, and for Γ, μmol mol^{-1}.

Data set	g_m	R_l	Γ	r^2
All	0.0675(±0.0040)	-1.85(±0.54)	27.4	0.8505
ψ_{xyl} > -0.70 MPa	0.0655(±0.0028)	-2.73(±0.41)	41.7	0.9625
ψ_{xyl} < -0.70 MPa	0.0793(±0.0058)	-2.29(±0.71)	28.9	0.8660

As this analysis is based upon a linear regression, it is possible to compare the slopes. The slopes (g_m) for the two different groups of potentials are significantly different at the 5% level. The slope for the data at the higher water potentials is smaller than that for the data at the lower potentials. This is not the response of A to water stress that had been expected (see below). Thus it was considered that there was no evidence for a direct effect of water potential on A, over the range of C_i within which the plants normally operate, despite the stomata closing in response to water potential by as much as 80%. To simplify further analysis, the straight line fitted through all the data, for values of C_i below 242 μmol mol^{-1} was used.

Using the mean value of C_a for all of the experiments in which the response to D was studied (334.8, s.e. ±1.2 μmol mol^{-1}) and the values of g_m and Γ derived from the linear regression, values of A were predicted from equation 8.4, using values of g_s predicted by equation 8.2, These are shown as a function of water potential, along with the 'raw' data in fig. 9.7. Predicted values of A as a function of D, for five levels of water potential, are also shown in fig. 9.8. The lowest water potential shown here is beyond the range used in the determination of the A/C_i curves. Thus the predicted curve may overestimate A if there is any direct effect of water potential on A. However, the magnitude of A is so small that, in comparison to the higher potentials, any additional effect of

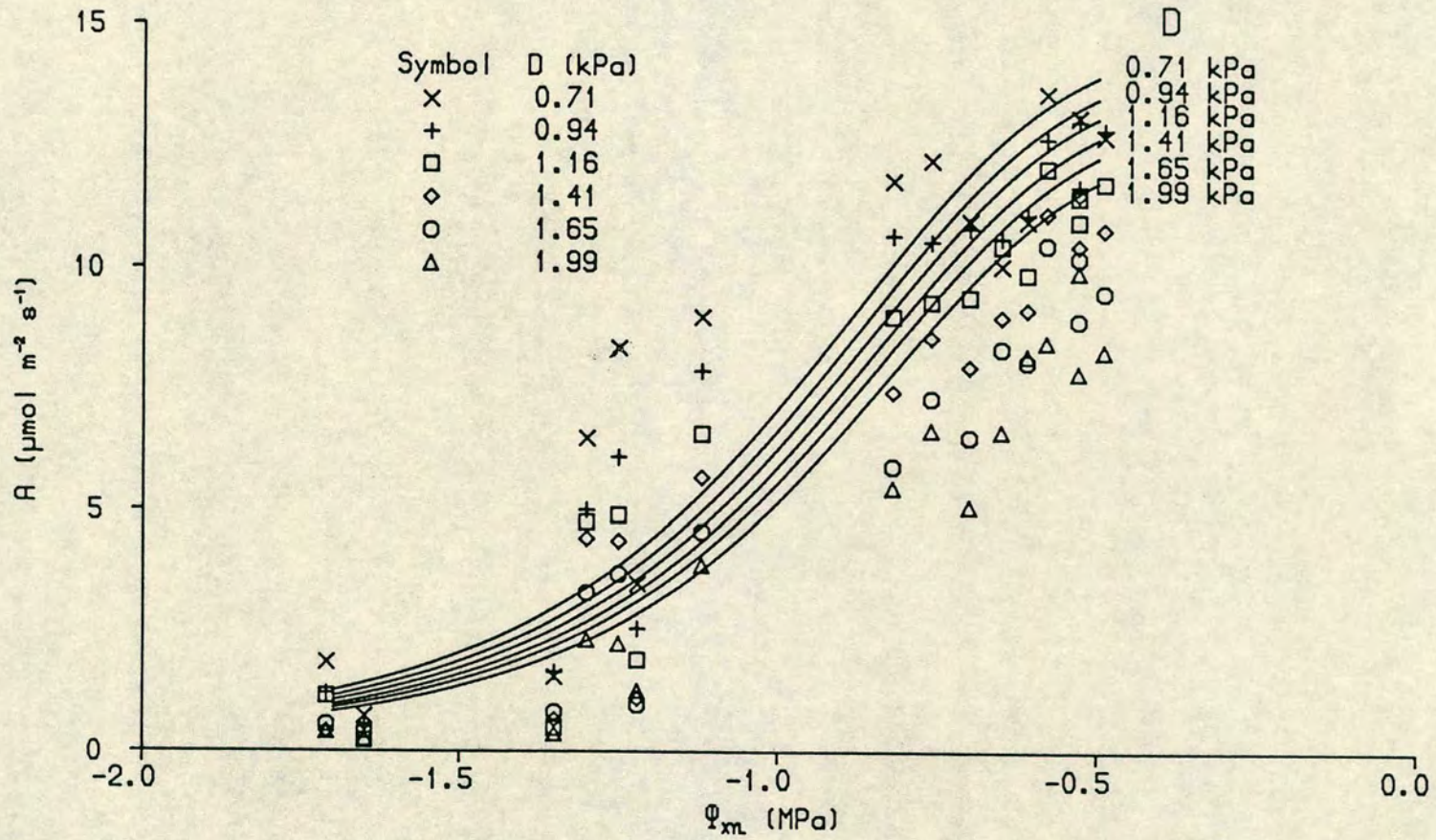

Figure 9.7: A as a function of water potential at 6 levels of D. The data points are pooled for 4 replicates. See the text for a description of the fitted curves.

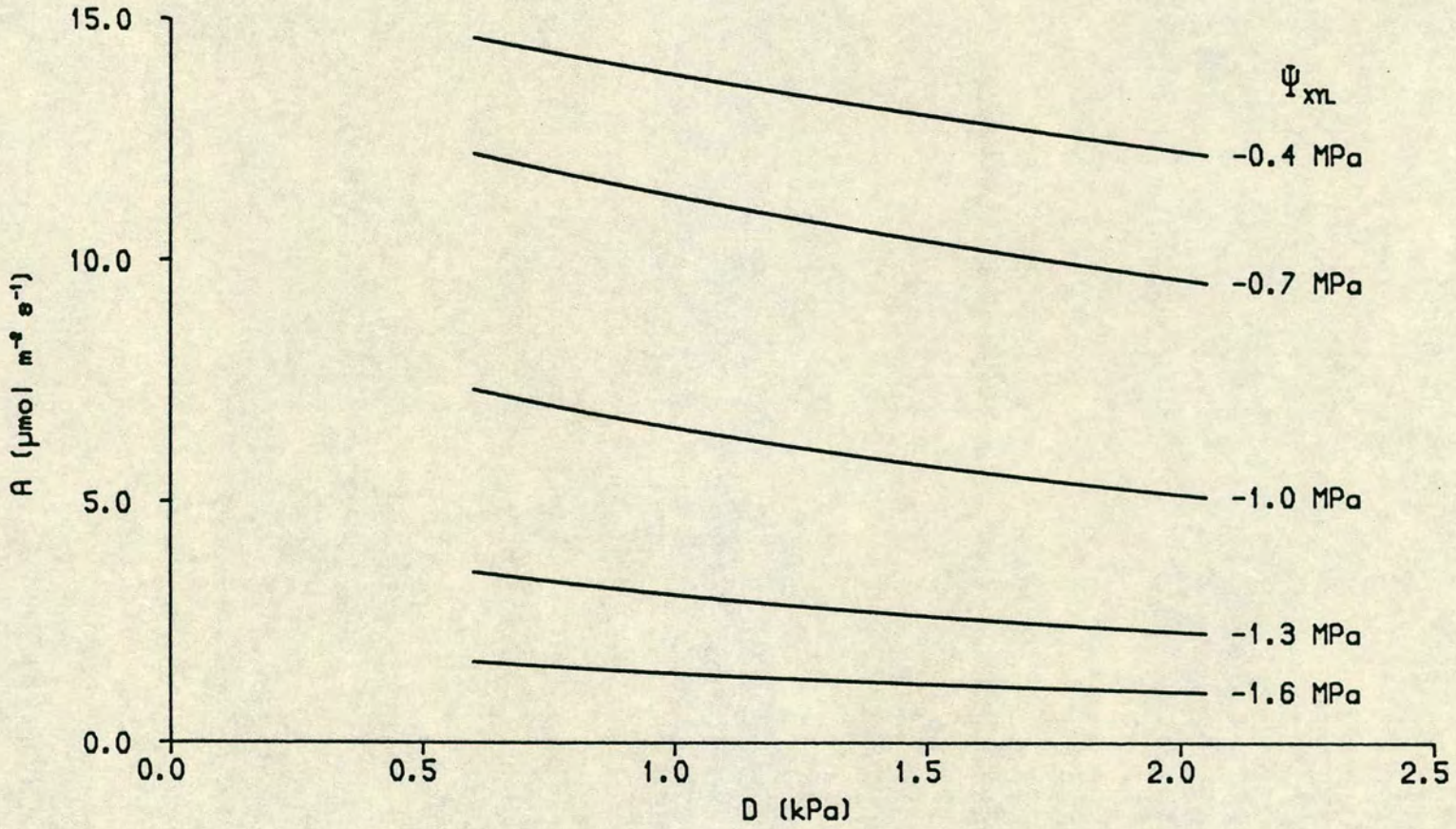

Figure 9.8: Predicted A as a function of D at 5 levels of water potential. See the text for a description of the fitted curves.

water stress on A will be minimal.

Having established that there is little direct effect of water potential on A, it was decided to test how the simpler A/g_s analysis, used in Chapter 8, would compare with the A/C_i analysis shown here. I thought it would be interesting to see if the A/g_s data would also lie on a common curve, or whether they would cross the curve as reported in Chapter 8. The data are plotted in fig. 9.9. The arrow indicates the trend for xylem water potential, associated with each experiment to decline. The solid curve represents the predicted values of A/g_s, using equation 8.4 with the parameters C_a and Γ, derived from the linear regressions, applied to the A/C_i data, described above. The dashed curve is equation 8.4 fitted to all of the A/g_s data. The derived value for g_m was 0.0562 (a.s.d. = ±0.0015) mol m^{-2} s^{-1}.

As the data for A/g_s also showed a trend to lie across the common fitted curves, the A/C_i data, from the experiments in which the responses to D were measured, are plotted in fig. 9.10. As the error in measuring C_i is large for small values of g_s and A, only the data for the first three days of measurement are plotted, i.e. those with a water potential > -1.2 MPa. As for fig. 9.9 the arrow indicates the trend for the water potential, associated with the data, to decline. The solid line is the straight line fitted to the A/C_i curve recorded at the start of each day. The dashed line is the line derived from fitting equation 8.4 to all of the A/g_s data.

If, over the range studied, there is no direct effect of water potential on A, it is clear that any reduction in A at lower water potentials, must, with our current understanding of CO_2 uptake, be attributable to stomatal closure. Several workers have proposed various methods of quantifying the degree of stomatal limitation (I_g) (Holmgren, 1965; Jones, 1973b; Farquhar & Sharkey, 1982). Applying such analyses to these data will not reveal a great deal more information about stomatal control of A. It is, however, interesting to test how such analyses cope with such data. Jones (1983) compared three methods of analysis. The simplest analysis originates from Holmgren (1965):

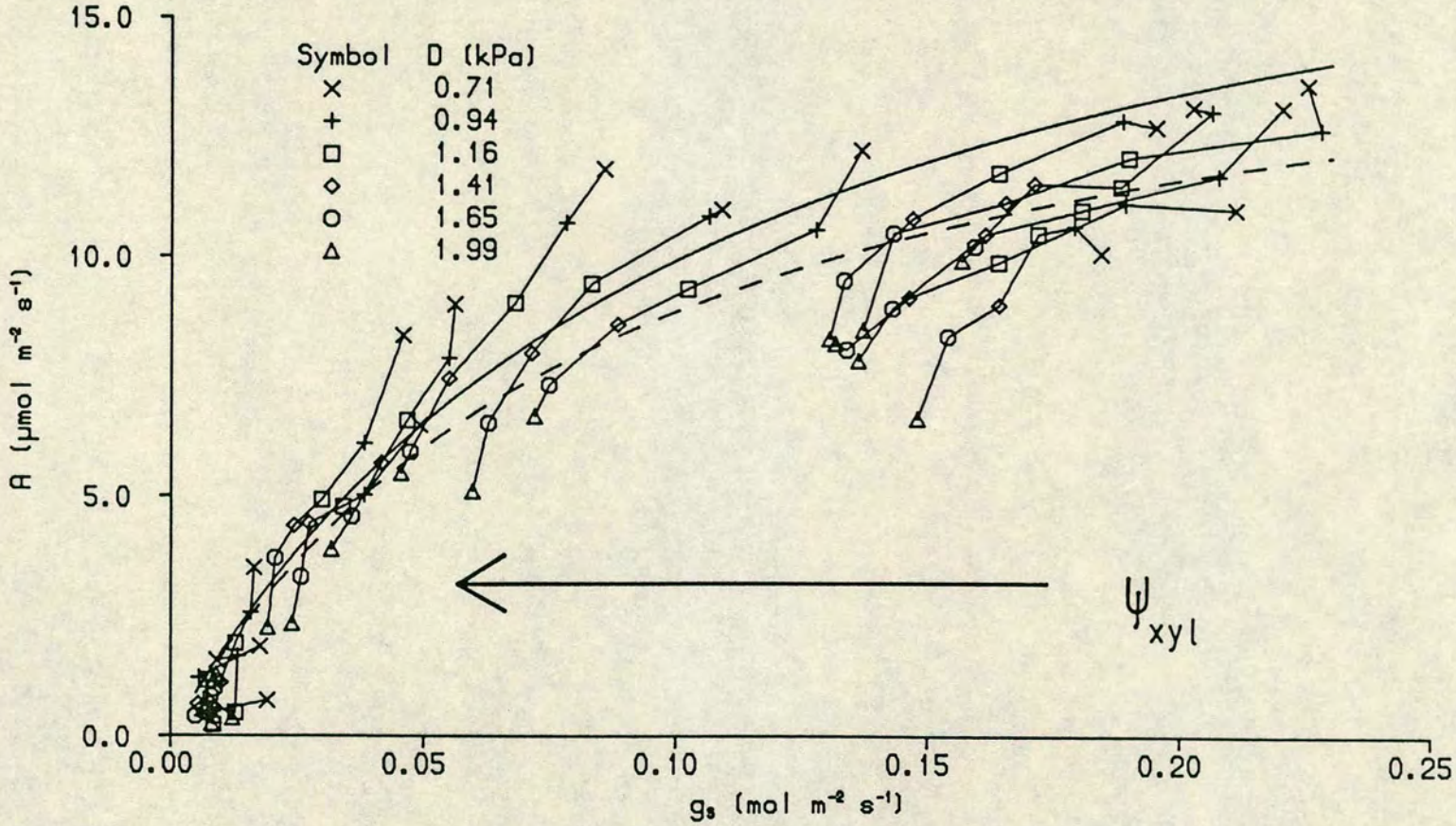

Figure 9.9: A as a function of g_s for all data. The data points for each day's 'D' experiment are joined by straight lines. The solid line was derived from the A/C_i measurements. The dashed line is fitted to the A/g_s data. See the text for additionals of the fitted curves.

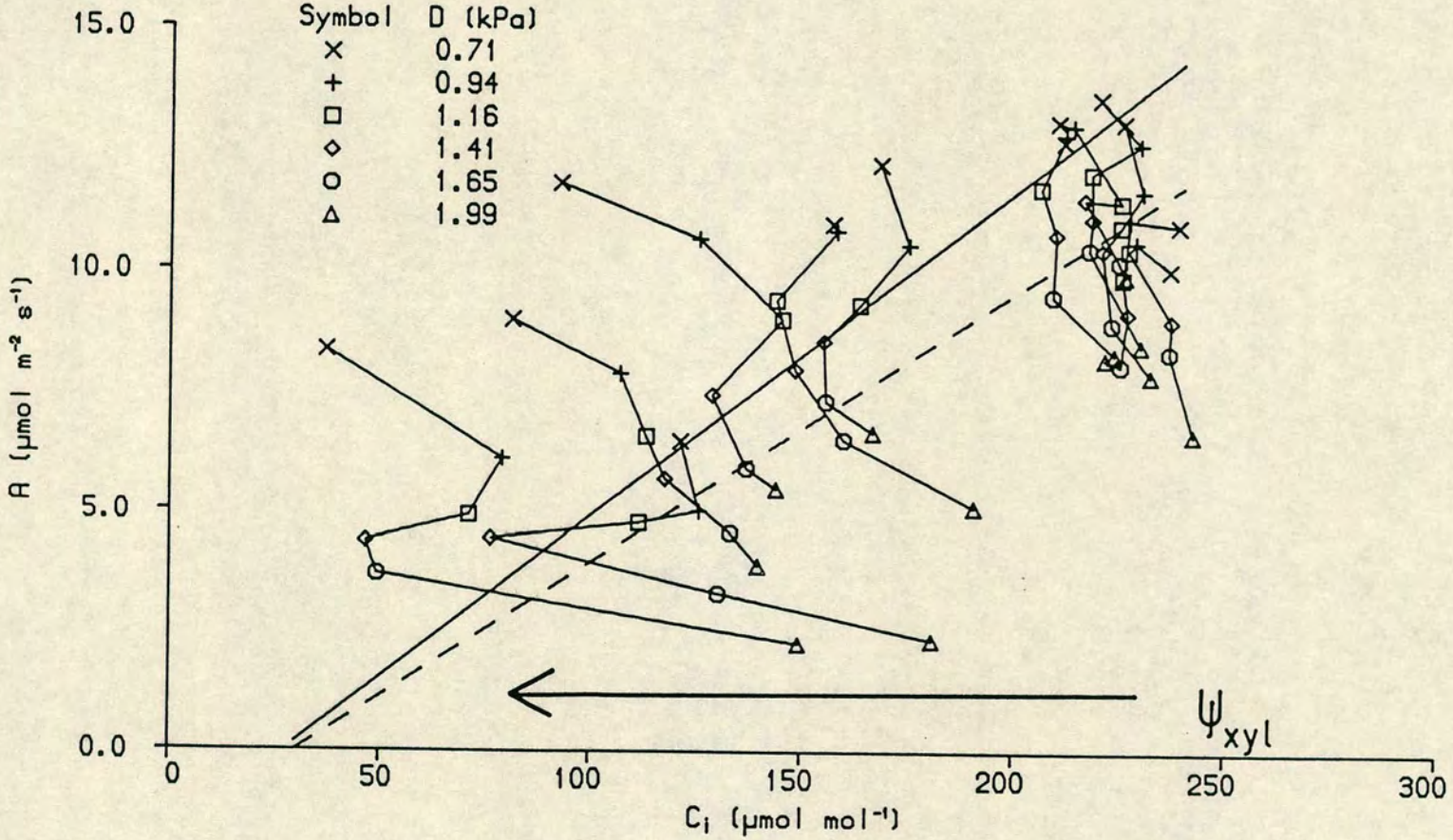

Figure 9.10: A as a function of C_i for selected data. The data points for each day's 'D' experiment are joined by straight lines. The solid line was derived from the A/C_i measurements. The dashed line was derived from the A/g_s data. See the text for additionals of the fitted curves.

$$l_g = r_{sc} / (r_{sc} + r_m) \qquad 9.6$$

This analysis is only really applicable to the linear 'RuP_2' saturated section of the A/C_i curve and further assumes that the A/C_i relationship is linear up to $C_i = C_a$, i.e. to $g_s = \infty$.

Jones (1983) suggests an alternative method:

$$l_g = r_{sc} / (r_{sc} + dC_i/dA) \qquad 9.7$$

This analysis is preferable to that described in equation 9.6, as it is applicable to situations in which the plant 'operates' on the non-linear section of the A/C_i curve. However, if the plant only 'operates' on the linear section of the curve, as shown for these data, dC_i/dA is equivalent to r_m and this analysis is identical to that above.

The third analysis was proposed by Farquhar & Sharkey (1982) and references A to a value A_o, which is the estimated value of A at $C_i = C_a$, i.e. at $g_s = \infty$. Thus:

$$l_g = (A_o - A) / A_o \qquad 9.8$$

As A/C_i was essentially linear over the working range of the plant, only Holmgren's analysis and the analysis of Farquhar & Sharkey were compared.

For the resistance analysis, r_{sc} was calculated as the inverse of g_{sc} as a function of D and water potential using the parameters fitted to equation 8.2. The value of r_m was calculated as the inverse of g_m derived from the common straight line fitted to the A/C_i data. For the Farquhar & Sharkey analysis, A_o was calculated by substituting the mean value of C_a, 335 μmol mol^{-1}, into the non-rectanglar hyperbola (equation 9.1) fitted to the A/C_i data. The corresponding value of A_o was 15.9 μmol m^{-2} s^{-1}. Predicted values of A were calculated, as described above, as a function of D and water potential. The two methods of calculating l_g are compared as a function of D, for four levels of water potential in fig. 9.11 and as a function of water potential for three levels of D in fig. 9.12.

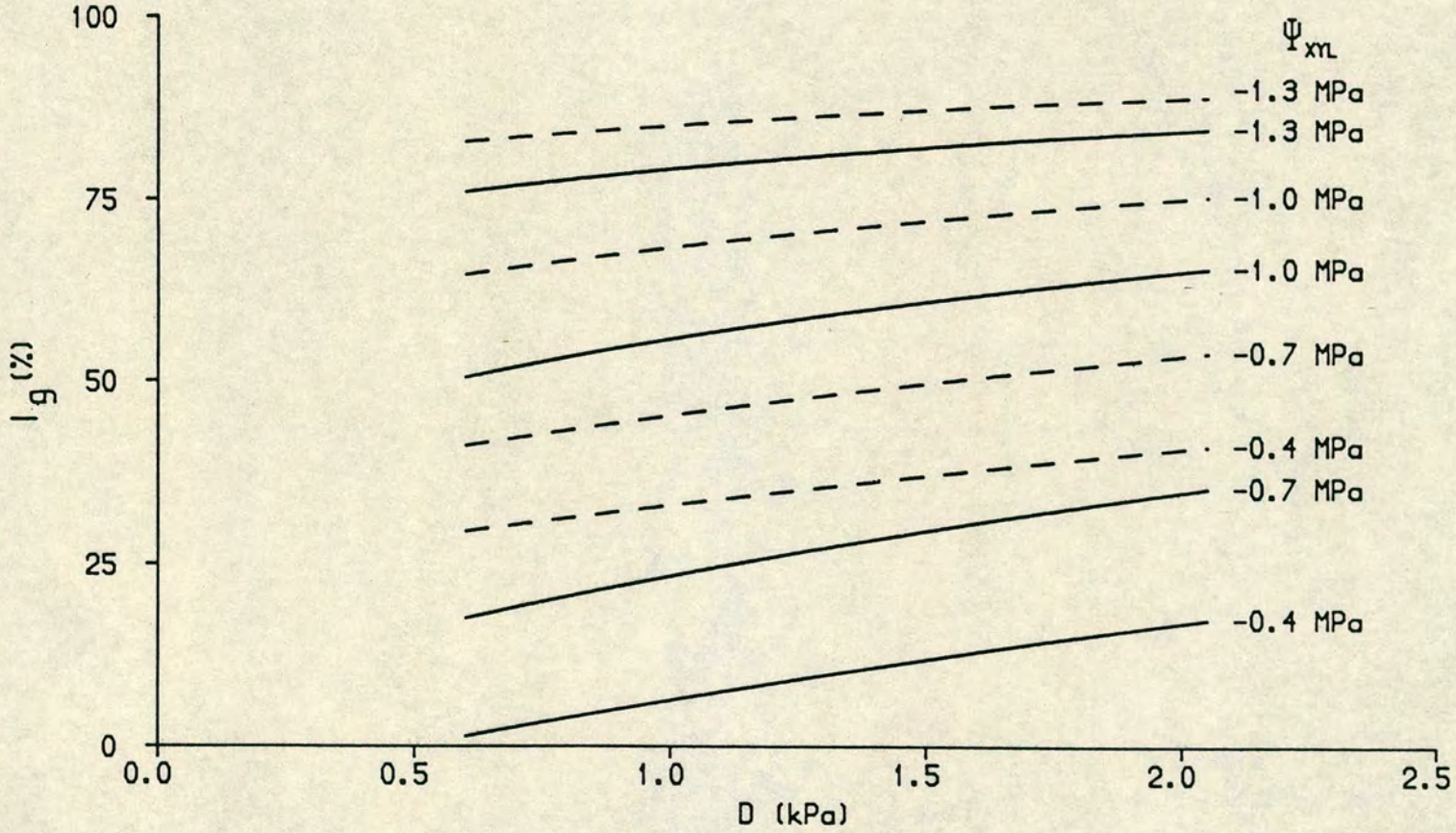

Figure 9.11: Predicted stomatal limitation, l_g as a function of D, at 4 levels of water potential. The dashed lines were calculated using a resistance analysis (see text) and the solid lines using the "Farquhar & Sharkey" method of estimating limitation.

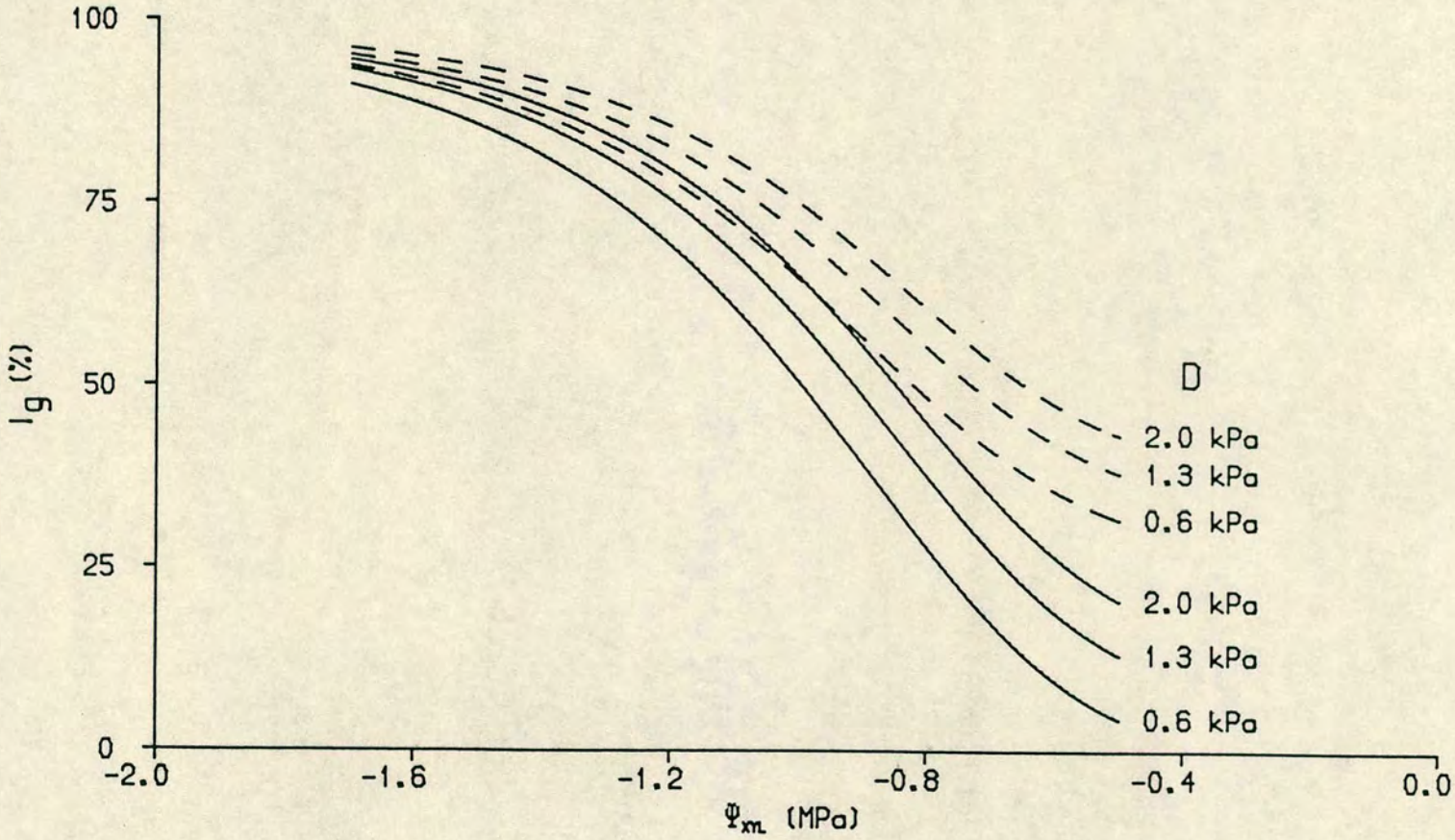

Figure 9.12: Predicted stomatal limitation, l_g as a function of water potential, for 3 levels of D. The dashed lines were calculated using a resistance analysis (see text) and the solid lines using the "Farquhar & Sharkey" method of estimating limitation.

As the model of A has been at least partially justified, by the A/C_i analysis, the models for E and A were combined to predict E/A. These predicted values are shown in fig. 9.13, as a function of D, for four water potentials.

As found in other experiments no significant change in xylem water potential was found between the measurements made at the start of the experiment and those at the end of each days experiment. The maximum difference for all the experiments was -0.09 MPa.

9.5 Discussion

The fit of the model (equation 8.2) to the g_s and E data appears to be quite reasonable (figures 9.1 & 9.2). Although not directly evident in these figures, much of the scatter around the fitted curves can be explained by variation in the response of the different replicates to water potential.

Unlike the experiment reported in Chapter 8, the fitted curves do not show a sigmoid response with respect to water potential. Thus a 'threshold' response is not shown. However, closer examination of the data shows that at high potentials there is some indication of a plateau (for g_s or E), but the data are distributed in such a way that the fit of the curves has not been influenced. More data would be required at higher potentials to test conclusively whether a threshold does or does not exist.

Parameter b of the model (see table 9.1) is more positive than found for either species studied in the experiments reported in Chapter 8. This implies that the stomata of these plants closed at slightly higher water potentials than in earlier experiments. Thus these data replicate the results, described in Chapter 8, which showed significant stomatal closure at much higher water potentials than have been reported previously for Sitka spruce.

It is possible that much of the variation in response to water potential, found in the literature, may be explained by differences in the

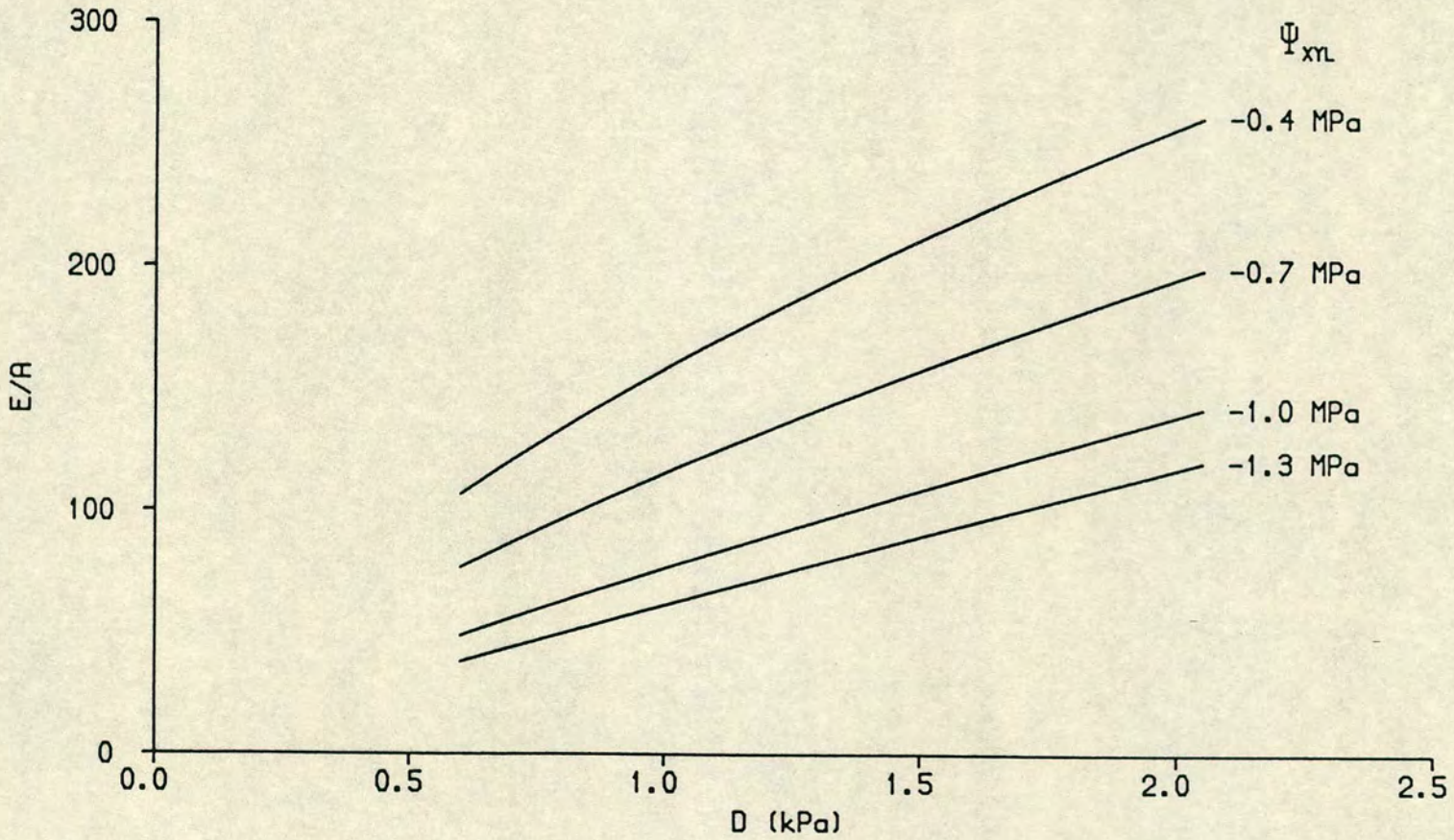

Figure 9.13: Predicted E/A as a function of D at 4 levels of water potential. See the text for a description of the fitted curves.

pretreatment conditions. A leaf, or even the guard cells alone, may have the capability to acclimatise, over a period of time, to different levels of water stress by osmotic adjustment (Jarvis, 1980). Thus for trees, in the field, which may normally suffer periods of mild or even severe water stress, one might expect a degree of osmotic adjustment and thus a lower threshold for stomatal closure compared to the plants used in these experiments, which were grown in well watered-conditions. The drying cycle imposed in these experiments also resulted in a faster drop in water potential than plants would probably experience in the field. Thus the plants in this experiment might not have had time to acclimatise osmotically, as they might in the field. The possibilty that these plants had abnormally low osmotic potentials was not tested.

It is unlikely that hypotheses of osmotic adustment can explain the difference between these results and those of Watts & Neilson (1978) who reported a much lower threshold for stomatal closure for similar Sitka spruce seedlings, stressed using a similar drying cycle. It is possible that their (unspecified) pretreatment conditions might have been so different as to cause a different response, or possibly that the difference is attributable to intra-provenance genetic differences. However, the high thresholds reported for Scots pine by Ng (1978) and in this thesis, were all on plants rooted in the U.C., peat-based, 'soil-less' compost, whilst Watts & Neilson used a John Innes compost. It is suspected that this could have been a major factor in determining the response.

Two properties of composts of the U.C.-type might influence the responses of the stomata.

1) The moisture release characteristics of peat-based soils (Boggie, 1970; Päivanen, 1973)) show a large proportion of water available to a plant, but below *ca* 20% water content, the soil water potential declines very rapidly for a small drop in water content and behaves in a similar manner to coarse sand (Örlander, in preparation). Thus the roots experience a very sudden drop in water potential, over a short time period. Although an attempt was made in this experiment to reduce this effect by replacing the bulk of the soil with a John Innes soil, the ball of peat, around the roots, might

act to mask the properties of the John Innes compost as the soil water conductivity for peat also decreases markedly at similar low water contents.

2) As a result of their high organic content, one of the characteristics of peat-based soils is physical shrinkage of the soil at low water contents. This, in addition to shrinkage of the roots (Huck *et al*, 1970), may drastically decrease contact between the roots and soil (Weatherley, 1976) and hence increase resistance to water movement. This will hasten the drop in root water potential.

Both of these phenomena will cause a moderately rapid drop in plant water potential when the soil reaches a low water content. However, the drop in the water potential at the root surface is likely to be much larger and faster than the consequent drop in bulk plant potential. There is now some evidence for the existence of a mechanism by which the roots sense low soil water potentials and can cause stomatal closure, independent of plant water potential status (Bates & Hall, 1981; R.Matyssek, in preparation). It is possible that the rapid, large decline in water potential at the root surface could suppress the production of cytokin-ins (Blackman & Davies, in preparation) which normally increase stomatal opening. This might have resulted in the stomata closing at higher leaf potentials than might be found for a different soil type.

The absolute values of g_s are approximately twice those found for the Sitka spruce plants used in Chapter 8. The values are similar to those in the plants used for the experiments described in Chapter 3. As these plants were also allowed to break bud 'naturally' this adds weight to the suggestion in Chapter 8 that the low conductances may have been a result of the 'forced' early bud break.

The response of g_s to D was, as shown for the plants in Chapter 8, less strong than reported for Sitka spruce in Chapter 3. The stomata closed by about 30% as D was increased from *ca* 0.6 to 2.0 kPa (figures 9.3 & 9.4). However, it should be noted that the range of D, for this experiment did not extend down to as low values of D as were used in the experiments in Chapter 3. As shown by the 'raw' data in fig. 9.2,

there was no sign of a decline in E at large values of D. Although not shown in this chapter, it should be realised that as shown in Chapter 3, dg_s/dD will decline as the stomata close both in response to D and to water potential, as this is inherent in the function used to describe the response of g_s to D. The relevance of this is discussed in Chapter 11.

The overall shape of the A/C_i response curves (fig. 9.5) are as predicted by the biochemical model of von Caemmerer & Farquhar (1981), i.e. at low C_i an initial linear increase in A with C_i referred to either as the 'linear, RuP_2 saturated' or the 'CO_2 limited' region of the curve. Above this the relationship 'curves over' to approach an asymptote, this section being known as the 'RuP_2 regeneration limited' region of the curve. The A/C_i curves for the low water potentials, at which g_s was only 20 - 50% of the values at the higher potentials, was not different to the curves for high potentials reported by previous workers.

There are two contrasting schools of thought as to how the A/C_i curve changes in response to water stress. Farquhar & Sharkey (1982) and Sharkey (1984) reported that the initial response to stress is a reduction in the asymptotic level, with little change in the slope of the linear region of the curve (otherwise known as g_m). Other workers have reported that both the slope and asymptote are reduced by moderate stress (Jones, 1983; Jones & Fanjul, 1983; Forseth & Ehleringer, 1983; J.Boyer, pers. comm.). In reality, it is probable that whilst initially there may be a reduction in the asymptote alone, as water potential declines, the slope of the linear region will, inevitably, also be reduced. The variation amongst the responses in the literature is possibly due to differences in the plant material and also ambiguity in the definitions of short and long term stress.

The data reported here show no trend for a decline in slope or asymptote, in fact quite the opposite. Whether one compares the initial slope of the non-linear relationship (table 9.2 & fig. 9.6), or the straight lines fitted to the A/C_i data, for C_i < 242 μmol mol^{-1}, the linear region of the curve has a steeper slope for the lower water potentials. The non-linear relationship also points to there being a higher asymptote at lower water potentials, although this must be treated with some caution

as there are fewer data for the lower potentials at high C_i.

It is possible to propose several hypothesis to explain the steeper slope for the linear region, at lower water potentials:

1) To achieve high potentials the soil was kept at 'field' (pot) capacity for much of the time. This may have resulted in waterlogging of the roots which might have disturbed the hormonal balance in the plant. This might act to cause a reduction in A. However, no evidence could be found in the literature for a response, where there was a reduction in A without any concurrent reduction in g_s. There is, however, some evidence for an optimum level of water potential for g_s, e.g. Jarvis & Jarvis (1963).

2) Assuming no effect of water potential on A, it is possible that the shoots were acclimatising to slightly higher photon flux densities in the assimilation chamber as compared to the pretreatment conditions. In addition the majority of the rest of the plant was subjected to darkness for one day in five. This will act to increase the 'sink' for photosynthesates produced in the shoot being studied. Similar effects have been shown to stimulate an increase in photosynthesis (see Jones, 1983, for discussion).

3) A third possible explanation is that there was a direct effect of changes in E on A as discussed in previous chapters. As a result of stomatal closure, E will be lower at the lower leaf water potentials. If E can reduce A directly, then one might expect higher values of A, at the same C_i, at the lower leaf water potentials (Sharkey, 1984).

Unfortunately, it is impossible to tell which of these explanations, if any, might have caused the change in slope.

It is interesting to compare the fitted parameters of the A/C_i curves in relation to the models used and the definition of mesophyll conductance. Looking at the values fitted for all water potentials pooled together (tables 9.2 & 9.3), the initial slope fitted for the

non-rectangular hyperbola is 35% higher than the fitted value for the straight lines. The value for Γ is also higher by a similar proportion. The difference is mainly due to the different form of the two models. The hyperbola is also biased by the data at high C_i and is therefore weighted by the data near the asymptote. This emphasises the point that one must be careful in interpreting such derived parameters without fully understanding the models on which they are based.

As to which model is most appropriate, i.e. which gives the 'right' values, this is dependent upon the use to be made of the results. The hyperbola is useful as it allows one to predict values of A from C_i over a wide range of C_i and in particular in the non-linear region, e.g the curve fitted to the data allowed the calculation of A_o (see above). The linear model, over the linear region of the A/C_i curve, is however, closer to the biochemical model of Farquhar *et al* (1980). This suggests that the slope is dependent on RuP_2 activity, which is virtually linear up to the point of curvature, although some slight curvature might be found as a result of increasing photorespiration. The linear relationship is also much simpler to use for modelling purposes (see above).

One must be careful in the use of terminolgy when discussing application of the linear model. If it is shown that the plant only operates on the linear region of the A/C_i curve, as shown for these data, then the slope is identical to the mesophyll conductance (derived as a residual resistance), as originally defined by Gaastra (1959). However, for many of the data in the literature, no test was made as to whether the plant operated on the linear region of the A/C_i curve. The resultant values of g_m, determined by residual resistance analysis, may therefore be erroneous (see Jones, 1983, for further discussion). Ideally the concept of mesophyll conductance should only be applied to the linear region, as this then allows it to be related to changes in the biochemistry of photosynthesis.

This must be borne in mind when comparing the values of g_m and Γ derived by use of the linear model here, with other studies. The value for g_m (0.0675 mol m^{-1} s^{-1}) falls within the range reported for conifers (Jarvis & Leverenz, 1983), but the value for Γ (27 µmol mol^{-1}) is somewhat

lower than that generally reported for conifers and other C3 plants. Some of the difference (*ca* 5 μmol mol^{-1}) may be accounted for by the inclusion of the 'Jarman correction' applied in this analysis (see Chapter 2), which has generally not been applied in previous studies.

As in Chapter 8 the individual A/g_s data points lie across the common line fitted through all of the data (fig. 9.9). The lower dashed curve, plotted by fitting equation 8.4, appears to fit the data reasonably well, confirming the A/C_i measurements which indicate that the plants operate on the linear region of the A/C_i curve. However, the derived value of g_m is some 15% smaller than the value derived for the A/C_i curve measured first thing in the morning. This difference is shown by comparing the solid and dashed curves in figures 9.9 and 9.10. The higher position of the solid line implies that the capability for photosynthesis declined during the course of the experiment.

Looking in detail at fig. 9.10, it is clear that in all of the experiments A declined with increasing D, despite C_i remaining constant or even increasing. The data for the lower values of C_i relate to measurements made on shoots with lower water potentials and smaller values of g_s. These data, although variable, because they are subject to large errors (see Chapter 2), show a distinct trend for C_i to increase as D was increased and g_s declined. As for the data in previous chapters, one possible explanation is that A can be directly affected by E. As these data, apparently provide the strongest evidence in this thesis for such a phenomenon, it is considered here in more detail.

The possibility that E may directly affect A was proposed by Sharkey (1984). Sharkey followed up the findings of several previous workers (Ball, 1981; Forseth & Ehlringer, 1983) who had shown, as above, in experiments in which D was the experimental variable, that A declined, despite $C/_i$ remaining constant or increasing. Forseth & Ehlringer also investigated this further and measured the A/C_i relationship at three levels of D for *Malvastrum rotundifolium* (Gray). For both studies E increased substantially with D because of only a slight response of g_s to D. Sharkey also studied A/C_i curves for a range of species and, in some experiments, 'checking' the calculated values of C_i using the direct method of measurement

(Sharkey *et al*, 1982). Like Forseth & Ehlringer, he found that as D was increased the asymptote of the A/C_i curve was reduced. He also found that this effect on A was not directly reversible, after D was decreased back to the starting level, even though the stomata recovered fully (cf. Chapter 4). Sharkey also showed that no significant drop in water content of the leaves was detectable, at high D, by use of a β-transmission gauge.

Although much of the effect, as seen by observation of the A/C_i curve, only occurs at values of C_i above the normal 'operating' range of these plants, Sharkey showed that several species (at least 9) exhibited a minimum response of a 10% reduction in A, caused by a 20% increase in E. In comparison, the data in fig. 9.10, for the higher potentials, show a decline of almost 30% in A at virtually constant C_i. The solid curve on this graph also lies close to the data points measured at a similar level of D to that imposed when the A/C_i curve was measured (1.1 kPa).

Using the evidence that increased E caused only small changes in leaf water content, Sharkey proposed that this phenomenom is probably a result of localised water stress in the mesophyll tissue caused by the higher E. He then extended his arguments, based on the the models of Tyree & Yianoulis (1980) and Sheriff (1982), to support the likelihood of large water potential gradients in the leaf. The models, however, apply to the gradient between the sites of evaporation, now thought to be close to the stomata, and the water conducting xylem tissue, i.e. not the photosynthetic mesophyll cells and are therefore not good supporting evidence for his hypothesis.

Following Sharkey's evidence, Bunce (unpublished) found similar results for *Chenopodium album*. He found a substantial reduction in A, for A/C_i curves measured over the range of C_i of 70 - 300 μmol mol^{-1}, when comparing curves determined at low and high D. The main difference between these responses and those of Sharkey were that *C. album* had a comparatively strong stomatal response to D, and as D was increased E remained either constant or possibly declined. Morison & Gifford (1983) found a similar trend in data for both *Oryza sativa* L. and *Phalaris aquatica* L. but they tentatively explained their results as a carry-over effect from the stepped changes in D (see Chapter 4). Bunce, however,

explained his results in terms of a direct response of A to D, although no mechanism was proposed to support this, probably because it is hard to envisage how the mesophyll can sense D, without changes in E.

Whilst Sharkey's findings appear to show more or less conclusively that E can directly affect A, the findings of Bunce and also of Morison and Gifford reveal that this phenomenon may not be so simply explained. The likelihood that many of these results might be caused by the imposition of stepped treatments of D is quite likely, as similar effects were also described in Chapter 4. It is also possible that, in addition, the techniques of measurement and assumptions made in defining C_i might also be causing part of this effect.

All of the studies, discussed above, use either Leuning's (1983) thoroughly derived method of calculation, or that of von Caemmerer & Farquhar (1981). However, both studies consider only boundary-layer and stomatal resistance to CO_2 fluxes, i.e. the resistance is considered to be from just inside the stomatal pore where most water is thought to evaporate. The internal resistance for CO_2 diffusion could be as high as 3.2 m^2 s mol^{-1} for *X. strumarium* (Farquhar & Raschke, 1978). Thus estimates of C_i may be slightly too high (Sharkey *et al*, 1982). Although this overestimate is small in relation to measurement errors, it is significant when looking at the small changes in A caused by changes in E. This resistance may also be much larger for other species, with different leaf structure, and is probably much larger for hypostomatous leaves. The molecular interactions between E and A, if internal resistance to CO_2 fluxes are significant, and the possibility that the site of E might change with g_s (Sheriff, 1979), all complicate the calculation of the CO_2 mole fraction at the mesophyll cell walls. Whether such an effect might be significant when E and D are manipulated requires more detailed analysis, outside the scope of this thesis, but such an effect cannot be ruled out as the cause of these phenomena.

The A/C_i curves measured each morning showed that there was no reduction in g_m even at water potentials down to -1.2 MPa, where the stomata were closed to only 25% of maximum g_s. Thus the hypothesis that localised stress might cause a reduction in A at large E, at constant

C_i, would, if one assumes that this stress affects A in a similar way to drops in bulk potential, require a drop in potential of at least 0.8 MPa to account for the *ca* 30% drop in A. As, unlike the epidermal tissue, mesophyll tissue is a large proportion of all leaf tissue, one would also expect to be able to detect this in the water potential measurements at the end of the day. Clearly further experiments are required to investigate the cause of this phenomenom.

As no hypothesis could conclusively explain these changes in the A/C_i relationship, I felt there was no basis for trying to include this in a model. Therefore all further analyses were done using the parameters for the linear model fitted to the A/C_i curve derived each morning, i.e. using the values of g_m and Γ given in table 9.3. This will inevitably result in an overestimation of A for the predicted curves involving A.

This explains the rather poor fit of the predicted A *versus* water potential curves to the data (fig. 9.7). Likewise the curve of predicted A *versus* D (fig. 9.8) may represent an overestimate of A. However, as in Chapter 8, the model gives curves of the response of A to D that are approximately linear, as found in all of the experiments described in previous chapters. The average slope of the line for - 0.4 MPa was *ca* -1.7 µmol m^{-2} s^{-1} kPa^{-1}. This value is within the range found for the four species, reported in Chapter 3, although this value is smaller than that reported there for Sitka spruce. This is probably the result of the weaker response of g_s to D for these plants. It can be seen that in both proportional and absolute terms the slopes of the predicted A/D curves decline as water potential declines. This reflects the response of the stomata alone and the model assumes that there is no direct effect of water potential on A.

The graphs of stomatal limitation (l_g) (figures 9.11 and 9.12) by both methods of analysis, show the inverse of the predicted response of g_s to D. Thus l_g increases with increasing D (fig. 9.11) and decreases with increased water potential (fig. 9.12). Comparison of the two methods of estimating limitation is interesting. Unlike the comparison of Jones & Fanjul (1983), there are marked differences between the methods. At the lowest D and highest water potential, stomatal limititation, as calculated

from the Farquhar & Sharkey (1982) equation (solid line), is very small and is lower for all levels of water potential in comparison for the limitation calculated using the resistance technique (dashed line).

The reason for this is that the resistance analysis effectively references A to a value of A obtained by extrapolating the straight line, whose slope is g_m, up to $C_i = C_a$, i.e. when $r_{sc} = 0$. This reference level is *ca* 20.7 μmol m^{-2} s^{-1} for these data, in comparison with 15.9 μmol m^{-2} s^{-1}, the value of A_o calculated from the non-rectangular hyperbola. The difference is a result of the curvature of the A/C_i curve between 250 and 340 μmol mol^{-1}, i.e. above the linear 'operating' range of the plant. Even the refinement of the resistance analysis proposed by Jones & Fanjul (1983), results in the same overestimate of l_g for data such as these. Therefore the 'Farquhar & Sharkey' technique gives a more realistic, quantitative estimate of stomatal limitation. However, as Jones (1983) pointed out, the reference point for A_o, as defined by Farquhar & Sharkey (1982), is rather hypothetical as a plant will never have infinite g_s and therefore never achieve A_o. Perhaps the major point that is evident from applying these analyses, is that one must have a full description of the A/C_i curve for the plants being studied before attempting to interpret the results of any limitation analysis.

It is possible to compare these results with the estimates of l_g for Sitka spruce, in response to water potential, made by Beadle *et al* (1981). Their analysis was based on the resistance technique, described above. They found that l_g was constant at 30% as water potential declined until the stomata were virtually closed. This differs from the results here as they found that g_s did not decline until much lower water potentials at which a direct effect of water potential on A was observed. The value for l_g at high potentials is similar though.

The predicted curves for E/A *versus* D (fig. 9.13) confirm the trends shown for the 'raw data' in Chapter 8. The curves, at high levels of water potential, show similar values to those in Chapter 3, i.e. E/A increases, more or less linearly with D, up to a value of 250 at 2.0 kPa. As water potential declines both the absolute value of E/A and the slope of the E/A *versus* D relationship decreases. Thus stomatal closure, whether

in response to D or water potential, causes a greater decline in E than A. This is as one would expect as E is directly proportional to g_s (equation 8.3) whilst A is not directly proportional to g_s, but becomes more dependent on the value of g_m as g_s gets smaller (equation 8.4). It should be noted that if g_m were reduced by low water potentials then the decrease in E/A would not be so great for the lower water potentials. This may have occurred at the lowest water potentials to which these plants were subjected, as indicated by needle yellowing, but this could not be quantified using the gas-exchange techniques here. However, under such conditions, preventing further water loss is important to the plant and A is so small anyway that E/A is not relevant. An analysis, similar to that outlined by Cowan (1977), of the stomatal response, predicted by the model for these data, which tests to see if this response is 'optimal' for minimising E/A, is presented in Chapter 10.

CHAPTER 10

STOMATAL FUNCTION IN RELATION TO WATER LOSS, ASSIMILATION AND CHANGES IN THE ENVIRONMENT

10.1 Introduction

The responses of g_s and A to various variables reported in this thesis have been obtained by controlling all other variables and studying the response to one particular variable, in isolation. One approach to find out how these responses affect a plant's survival in the field is to develop complex models of the plant's physiology, using parameters derived from experiments such as those reported here, and then to integrate the output over a period of time to give total dry matter production and total water loss. However, such a procedure can be extremely complicated and time consuming and it is often not easy, from the results, to determine the exact contribution of any one particular response to the end result.

In a series of papers, Cowan (1977, 1978, 1982) and Cowan & Farquhar (1977) proposed an alternative approach. Taking a "top down" approach they asked the teleological question of how should the stomata respond to changes in the environment? They developed this approach by assuming that the role of the stomata is, over the course of a day, to minimise the average amount of water lost, whilst at the same time minimising stomatal limitation of A. Thus, over a period of time (t), assuming

$$\int_0^t A \, dt = \text{a constant}$$

the stomata act to minimise

$$\int_0^t E \, dt.$$

By applying the technique of calculus of variations, it can be shown that $(dE/dg_s)/(dA/dg_s)$ should be a constant, for any given total of A, to optimise the above hypothesis, i.e.

$$dE/dA = \lambda = \text{a constant,} \qquad 10.1$$

providing that $(d^2E/dA^2) > 0$.

A similar concept has been applied to the balance between photosynthetic and non-photosynthetic tissue (Cowan, 1979), to plant competition (Cowan, 1982) and to plant growth form (Schulze, 1982). The hypothesis has also been extended to cover CAM plants (Farquhar & Sharkey, 1982) which cannot maintain dE/dA constant in the short term, because they ultimately fix CO_2 during the day when the stomata are closed. Thus, since the original hypothesis was developed for stomata, the ideas have been expanded.

Although one might expect the stomata to behave in an "optimal" way, as this should be of a selective advantage with respect to evolution, thorough tests of the stomatal concept, let alone the later hypotheses, have not been made. One of the reasons for this is that it is very hard to design an experiment to test whether all of the responses of the stomata and all possible interactions of these responses tend to maintain dE/dA constant. In addition, in the field, a plant is rarely in a steady-state condition so one must also consider the dynamic responses of the stomata to changes in the environment, as these responses may, in a very variable environment, be most important in determining the daily total of E with respect to the daily total of A.

Attempts to confirm the optimisation hypothesis to date fall into two groups. Firstly, there are experiments designed to measure the response of g_s and A to either D or T, for which the data have been analysed, to see if dE/dA remains constant, as the stomata respond to the independent variable (Farquhar *et al*, 1980b; Hall & Schulze, 1980; Field *et al*, 1982; Meizner, 1982). Secondly there are field studies in which the timecourse of g_s and A have been monitored over a period of time and used to calculate dE/dA, e.g *Rhamnus californica* Esch. (Williams, 1983).

According to Cowan (1982) all of the controlled environment experiments, referred to above, demonstrated conservation in dE/dA as D was changed. However, the definition of "conservation" has been applied

fairly flexibly, for example Meizner (1982), for Douglas-fir, reported a constant value of dE/dA which, for one replicate, declined by 45% as D was increased from 0.6 kPA to 1.8 kPa at 25 °C. Similarly the results of Farquhar *et al* (1980b) for *Corylus avellana* L., at low water potentials, showed that dE/dA increased by up to 200% as D was increased from *ca* 0.5 to 3.0 kPa at 28 °C. Hall & Schulze (1980), for *Vigna unguiculata* L., also showed similar deviations from constancy at low water potentials.

Williams (1983) found that during the timecourse of a day the stomata did not respond in a way that maintained dE/dA constant all of the time. They only responded in this way during the middle of the day when the potential for E and for A was highest. During the early morning and late evening dE/dA changed drastically, but under such conditions E and A were small anyway so in terms of the daily integrals of E and A, these deviations are likely to be insignificant. He did, however, find that the actual daily integrals of E and A did not differ significantly (for $p > 0.05$) from the totals predicted if the stomata had behaved "optimally".

The majority of studies to date have treated D as the independent variable to test the hypothesis. The reason for this is that Cowan (1977) predicted, using a simple linear A/C_i model, that under many conditions, for small values of λ, a strong stomatal response to D was required to maintain dE/dA constant. Such a response was required to account for mid-day closure of stomata.

Therefore I decided to see if the responses of g_s and A to D, that I had measured, resulted in dE/dA remaining constant. In particular, I was interested to see if the model of g_s and A as a function of D and water potential, described in Chapters 8 & 9, would result in dE/dA remaining constant.

10.2 **Theory**

Various methods have been used to calculate dE/dA. The original equations derived by Cowan (1977) were for a leaf "standing free" in the natural environment. Under such conditions any factor which acts to cause

a change in E would also change the energy balance of the leaf, in particular leaf temperature. Terms were included in the equations to account for such effects, with respect to both the calculation of dE/dg_s and the calculation of dA/dg_s, the latter being affected as a result of the sensitivity of A to changes in leaf temperature.

In a well-ventilated, temperature-controlled assimilation chamber, as used here, these secondary terms become small and the equations can be simplified (Farquhar *et al*, 1980b; Meizner, 1982). However, it should be realised that by placing a leaf in such a chamber, the microclimate of the leaf is totally artificial.

In the natural environment, a change in g_s will usually cause a change in leaf temperature because of an effect on the latent heat term in the energy balance of the leaf. The change in temperature may also affect A. In contrast, in the assimilation chamber, the leaf is prevented from changing temperature. Similar arguments can be developed for D, as, in the chamber, changes in E caused by changes in g_s do not have any effect on D, as D is controlled to a fixed value. In the field, for plants with large boundary layers, e.g. plants in a dense canopy, an increase in E will cause the value of D around the leaf, to decrease to a degree. Thus it is unlikely that the response of g_s to D, and the calculated values dE/dA, measured in an assimilation chamber will be identical to those for the plant in the field.

Nonetheless, because of the leaf size and tree structure, the boundary layer conductance of conifers is generally fairly large in the field. Thus changes in temperature and D, when E changes, are small compared to broad leaf species and the environment of the chamber is not likely to result in totally unnatural responses.

dE/dA can be found as the ratio of dE/dg_s and dA/g_s. If the energy balance corrections are ignored, on the assumption that the boundary layer is small, then dE/dg_s can be found by differentiating:

$$E = g_s D / P = \Delta w \, g_s . \qquad 10.2$$

Thus

$$\frac{dE}{dg_s} = \frac{D}{P} = \Delta w \qquad 10.3$$

To determine dA/dg_s requires A to be expressed as a function of g_s. The best way to do this is to determine the A/C_i relationship (Farquhar *et al*, 1980b). As this was not done in the experiments described in Chapters 3, 5 and 7, a simpler approach must be followed. For all these data g_s was found as a function of D using equation 3.3. A was also found as a function of D using a linear regression (see tables 3.3, 5.2 and 7.2). By converting all units to their base units, solving equation 3.3 for D and then substituting the result into the linear regression for A, one arrives at the following relationship:

$$A = m E_m P / g_s - m E_m / a + A_i \qquad 10.4$$

where m is the slope of the A/D curve and A_i the intercept at D=0.

It is then possible to differentiate this to give:

$$\frac{dA}{dg_s} = \frac{-m P E_m}{g_s^2} \qquad 10.5$$

Thus by combining equations 10.3 and 10.5, the solution for dE/dA is:

$$\frac{dE}{dA} = \frac{D g_s^2}{-m P^2 E_m} \qquad 10.6$$

Therefore it is possible to find dE/dA for the data in Chapters 3, 5 and 7 using a purely descriptive model for A as a function of g_s.

The data presented in Chapter 8 and 9, are described by equation 8.4 which gives A as a function of g_s. By converting all units to their base units and differentiating this equation one gets:

$$\frac{dA}{dg_s} = \frac{(C_a - \Gamma)\, g_m^2}{(g_s/1.6 + g_m)^2} \qquad 10.7$$

By combining equation 10.3 and 10.7, the equation for dE/dA is thus:

$$\frac{dE}{dA} = \frac{D\,(g_s/1.6 + g_m)^2}{P\,(C_a - \Gamma)\, g_m^2} \qquad 10.8$$

10.3 **Results**

Using the above equations, dE/dA was calculated for the data described in previous chapters. It can be seen that both equations 10.6 and 10.8 give dE/dA as a function of g_s. To generate a curve for dE/dA, with D as the independent variable, the relevant models for g_s as a function of D were used to calculate the values of g_s in these equations.

For the results described in Chapters 3, 5 and 7, the model for g_s of the form of equation 3.3 was used, with the parameters E_m and a, fitted for each experiment. The resultant graphs of dA/dg_s and dE/dA as a function of D are shown, respectively in figures 10.1a+b for Chapter 3, 10.2a+b for Chapter 5 and 10.3a+b for Chapter 7.

For the results described in Chapter 9, the model for g_s as a function of D and water potential of the form of equation 8.2 was used, with the parameters E_m, a, b and c, fitted for that experiment. Graphs of dA/dg_s and dE/dA as a function of D, for four levels of water potential, are shown in figures 10.4a and 10.4b, respectively. Similar plots were found using the parameters for the experiments in Chapter 8, but are not presented as the use of equation 8.4 was not fully justified by determination of an A/C_i relationship, as was done in Chapter 9.

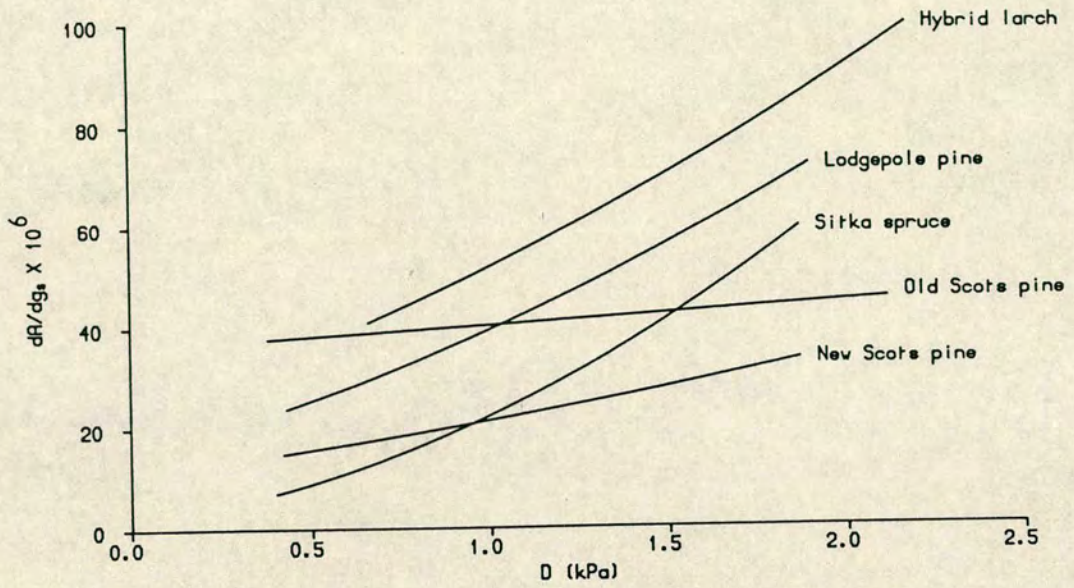

Figure 10.1a: dA/g_s as a function of D, for four species of conifers. See the text for a description of the curves. The parameters were derived from the data described in Chapter 3.

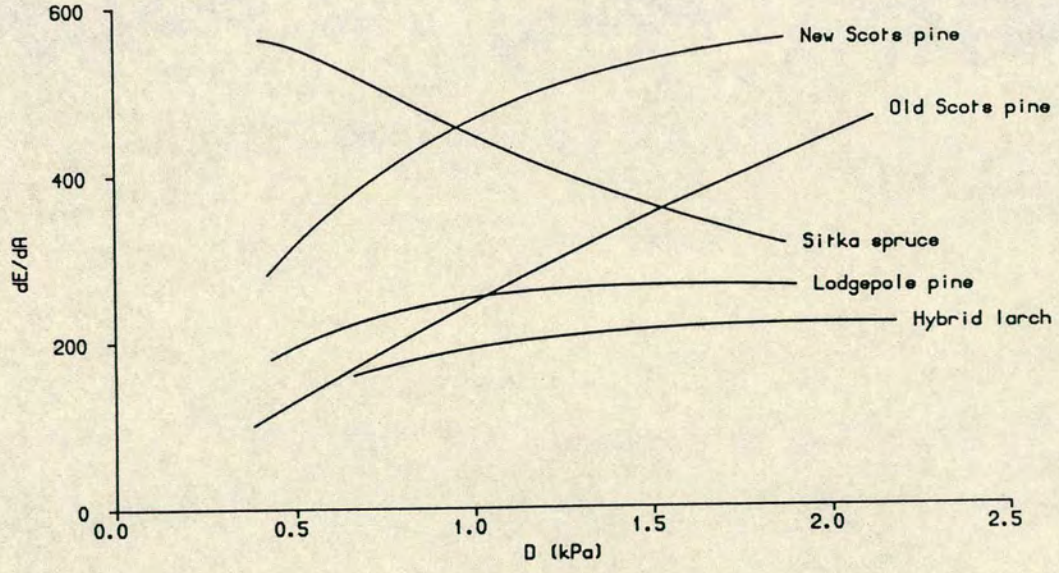

Figure 10.1b: dE/dA as a function of D, for four species of conifers. See the text for a description of the curves. The parameters were derived from the data described in Chapter 3.

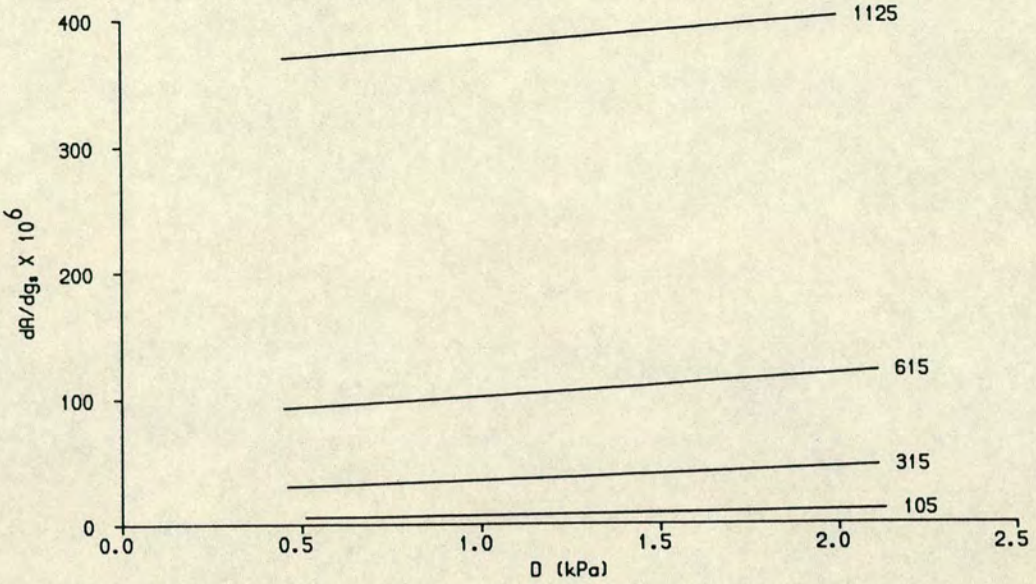

Figure 10.2a: dA/g_s as a function of D, for four levels of Q (μmol m^{-2} s^{-1}). See the text for a description of the curves. The parameters were derived from the data described in Chapter 5.

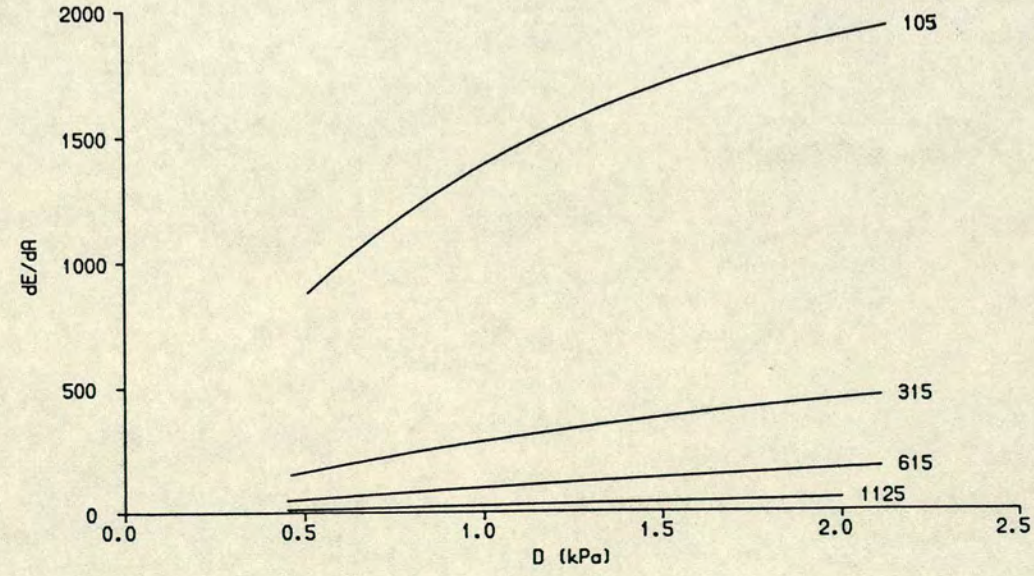

Figure 10.2b: dE/dA as a function of D, for four levels of Q (umol m^{-2} s^{-1}). See the text for a description of the curves. The parameters were derived from the data described in Chapter 5.

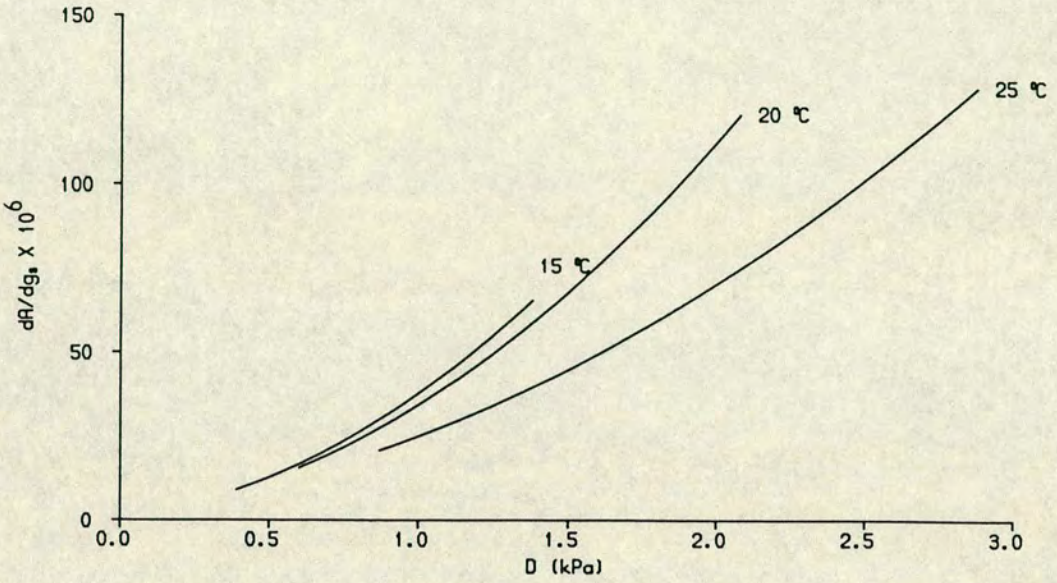

Figure 10.3a: dA/g_s as a function of D, for three temperatures. See the text for a description of the curves. The parameters were derived from the data described in Chapter 7.

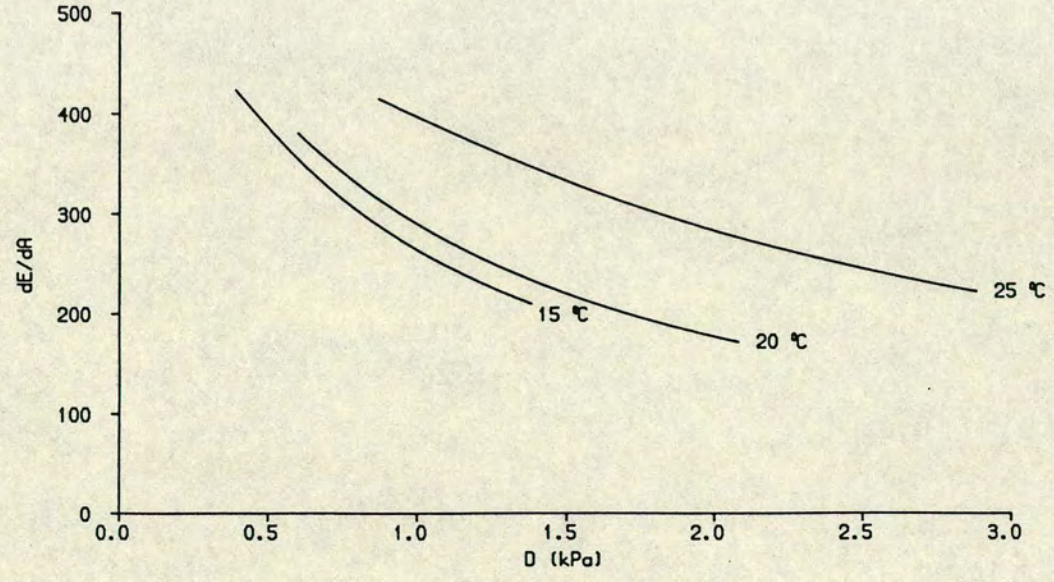

Figure 10.3b: dE/dA as a function of D, for three temperatures. See the text for a description of the curves. The parameters were derived from the data described in Chapter 7.

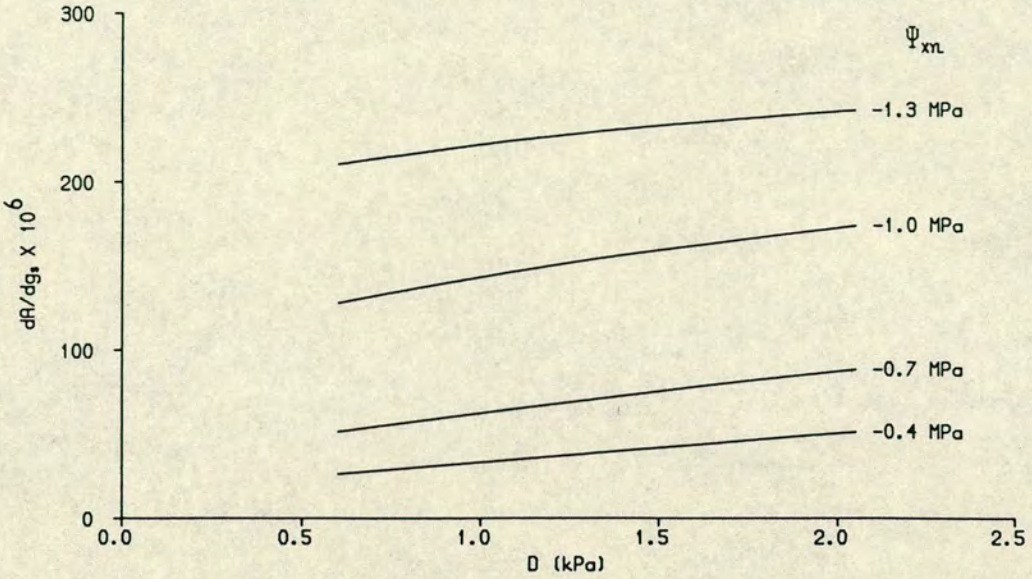

Figure 10.4a: dA/g_s as a function of D, for four levels of water potential. See the text for a description of the curves. The parameters were derived from the data described in Chapter 9.

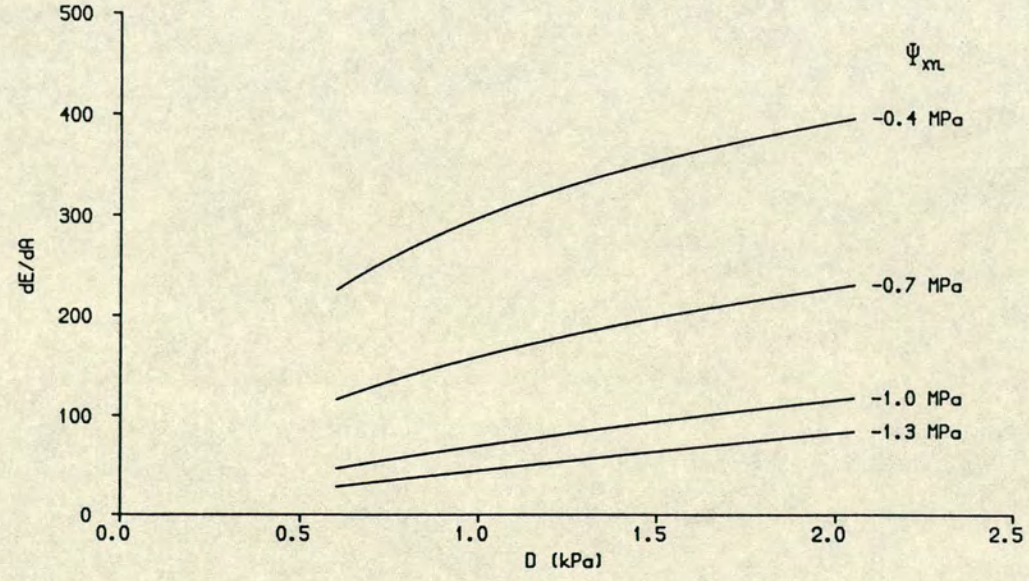

Figure 10.4b: dE/dA as a function of D, for four levels of water potential. See the text for a description of the curves. The parameters were derived from the data described in Chapter 9.

If dE/dA is to remain constant, following the assumption made above that $dE/dg_s = \Delta w$, then dA/dg_s should increase linearly with D. Both graphs are given to aid interpretation.

For all of these graphs, only the resultant continuous functions are shown over the range of D within which g_s and A were measured. It was felt that plotting "data points" on these curves, as done by many workers presenting similar results, is misleading. Such points can be generated by substituting measured values of g_s, at a known level of D, into an equation of the form of 10.6 or 10.8, to find a value of dE/dA. However, as shown above, a fitted relationship between A and g_s is required to find some of the parameters in these equations. Thus more variation underlies the derivation of the points than is apparent from their distribution around a continuous curve. Extending these arguments further, it can be seen that estimation of the error associated with any calculated value of dE/dA, i.e. for "data points" or continuous functions, is virtually impossible as the parameters required for the derivation of dE/dA are the result of fitting two, independent non-linear relationships. The interpretation of such curves must be approached with caution although some estimation of when the errors are likely to be large can be made.

For the results in Chapters 3, 5 and 7 the determination of dA/dg_s was done indirectly using the A *versus* D data measured in each experiment. As, in some cases, g_s was not very sensitive to D, the range of values of A and g_s was very limited, so that the estimation of the slope will be subject to very large errors. Similar errors are discussed by Farquhar *et al* (1980b). Thus the estimation of dE/dA for the old Scots pine shoots in Chapter 3 and all of the results for Chapter 5, must be considered with added caution. In addition, there was a decline in A, reported in Chapter 5, which could not be attributed to stomatal closure. This will also affect the estimate of dA/dg_s.

For more precise determination of dA/dg_s, the A/C_i relationship should be determined. This was done in Chapter 9, where the errors associated with the determination of dA/dg_s are likely to be smaller than in other chapters. As the A/C_i relationship was adequately described by a linear

model over the "operational" range the equations derived for calculating dE/dA are simplified versions of those presented by Cowan (1977), who also assumed a linear A/C_i model. Cowan used his model, with simulated climatic data, to predict the daily course of g_s for constant values of dE/dA. Many workers have used these predicted courses of g_s as indirect evidence for a direct response to D, therefore I have used equation 10.8 to see what the response of g_s, E and A to D would be if dE/dA was constant, using the physiological parameters derived in Chapter 9 for Sitka spruce.

This was done by solving equation 10.8 for g_s. Then, using the values of g_m, C_a and Γ found in Chapter 9, g_s was calculated over the range of D studied in Chapter 9. E was found using equation 10.2 and A was calculated by substituting the calculated values of g_s into equation 8.4. The predicted curves for g_s, E and A as a function of D for values of λ of 100, 200, 300, 400 and 500 are shown in figures 10.5, 10.6 and 10.7 respectively. This range of λ was chosen as it covers the range found for the data described in chapters 3, 7 and 9. The dashed lines on the curves represent extrapolation beyond the range of values studied in Chapter 9.

10.4 Discussion

The graphs relating to the data in Chapter 3 (figures 10.1a+b), show a range of responses of dA/dg_s and dE/dA. All of the curves for dA/dg_s increase with D, although the curve for old Scots pine has a very small slope. Only the curves of dE/dA for lodgepole pine and hybrid larch can really be considered to show a constant value. It is interesting that Sitka spruce, which had the strongest stomatal response to D, shows a very steep decline of dE/dA with D, whilst lodgepole pine and hybrid larch, which only had moderate responses of g_s to D (see fig. 3.1), show much smaller changes. Thus a strong response of g_s to D is not essential for dE/dA to be constant.

Both ages of Scots pine shoots show a similar trend for dE/dA to increase with D, although the absolute values are different. However, because of the very weak stomatal response to D, of the old Scots pine shoots, determination of this dE/dA curve is prone to very large errors

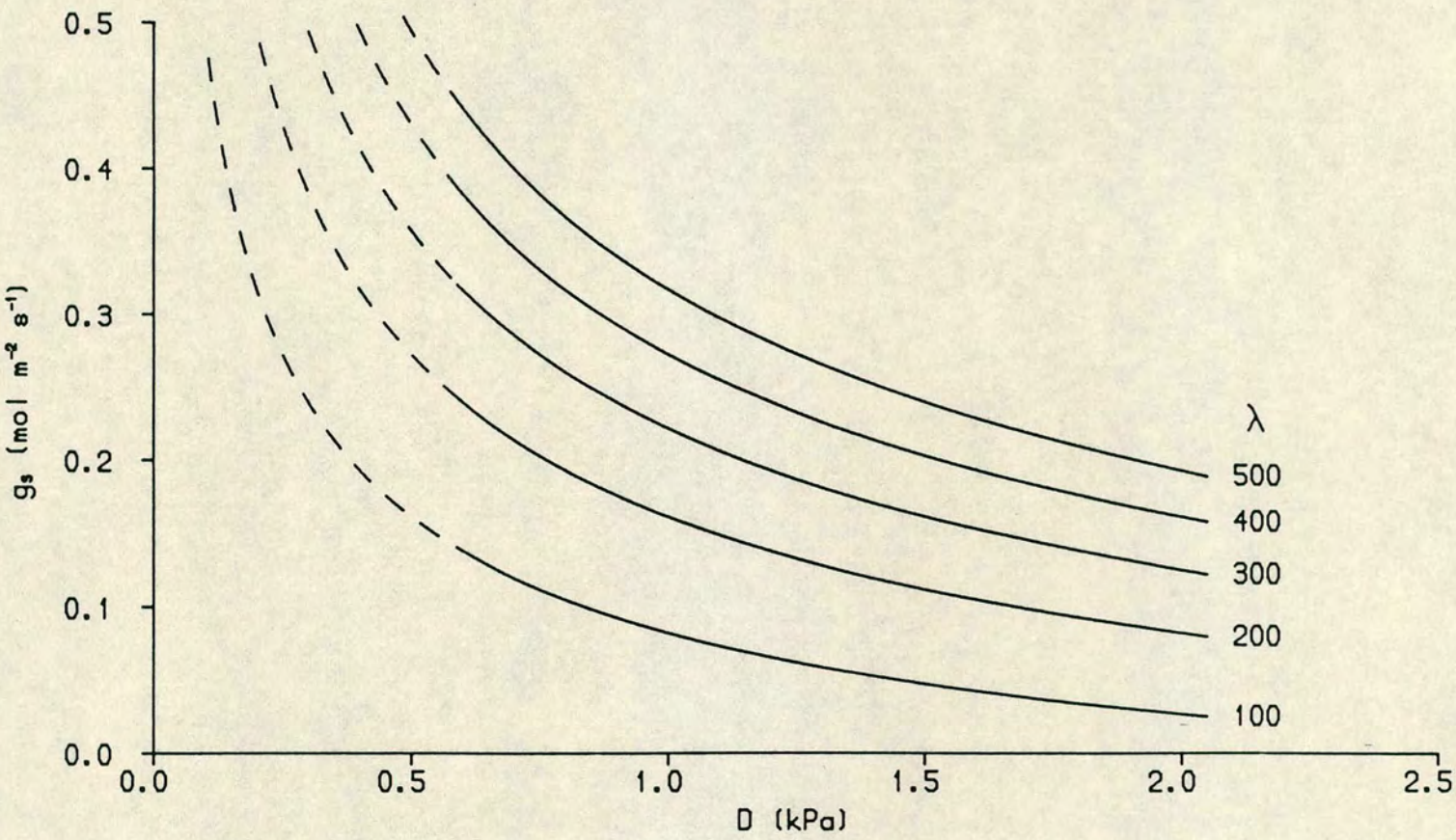

Figure 10.5: g_s as a function of D, for four levels of dE/dA. See the text for a description of the curves.

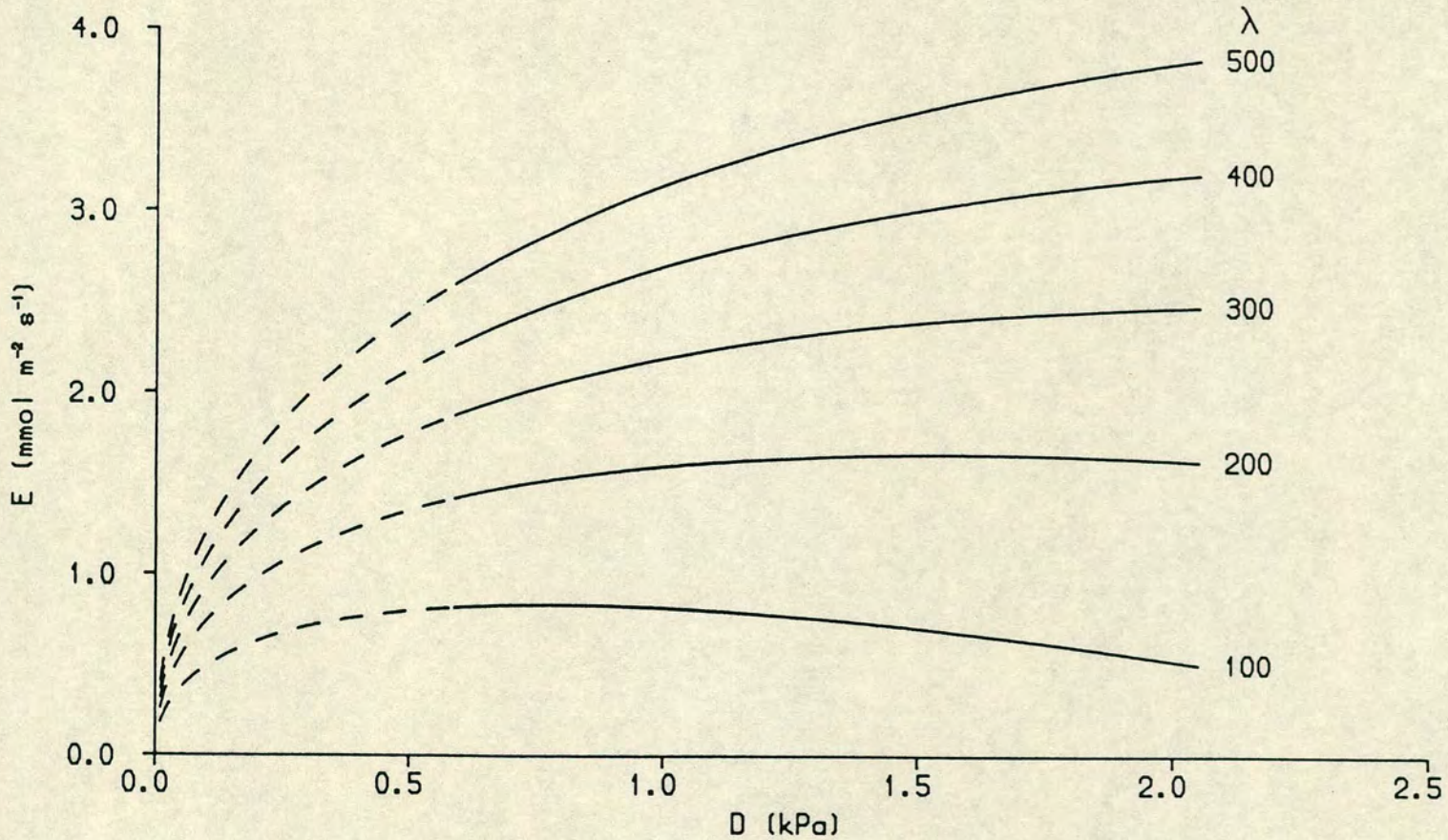

Figure 10.6: E as a function of D, for four levels of dE/dA. See the text for a description of the curves.

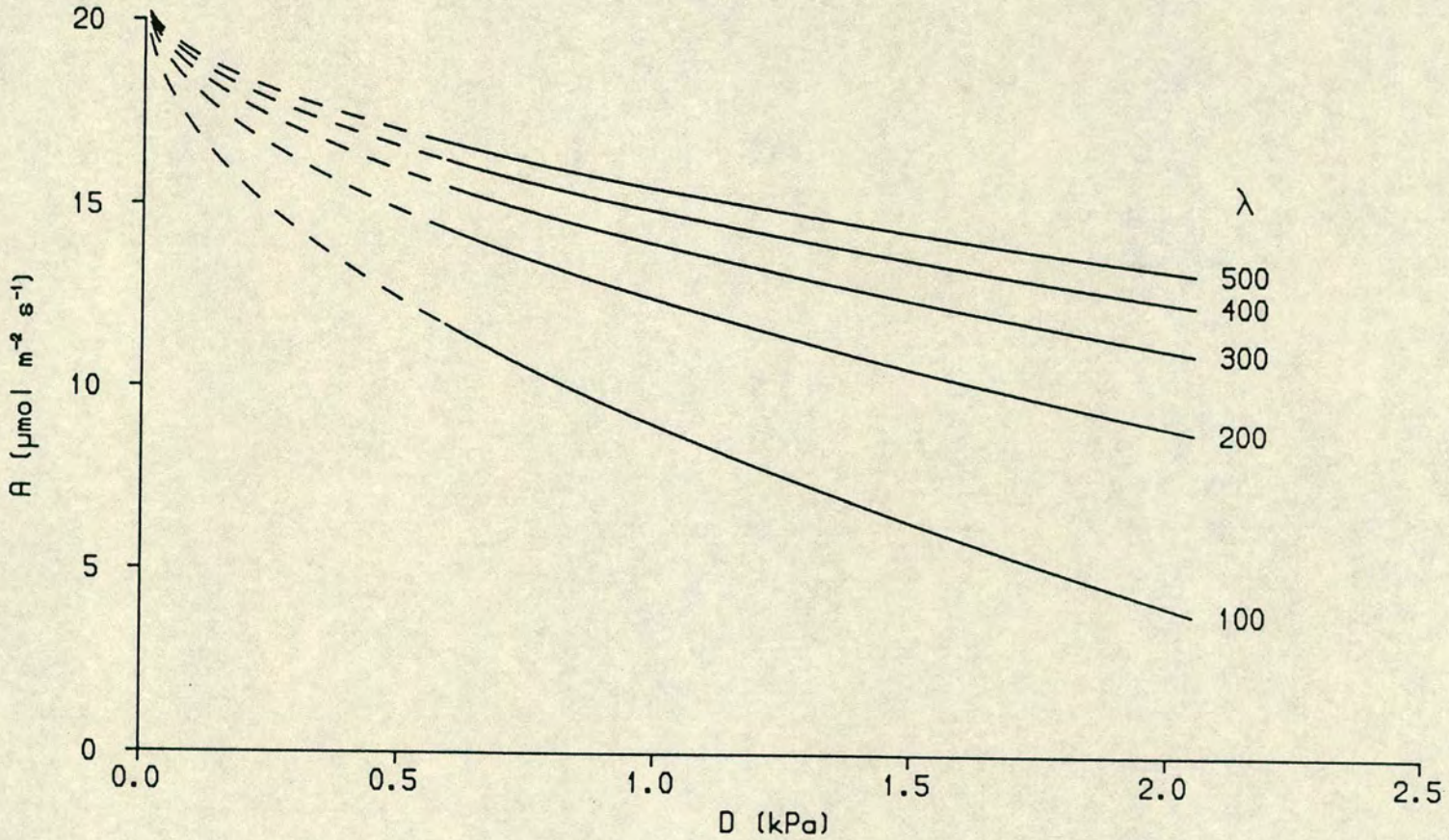

Figure 10.7: A as a function of D, for four levels of dE/dA. See the text for a description of the curves.

(see above), so no real conclusions can be drawn from the comparison.

Similar arguments, with regard to errors, can also be applied to the curves for the data relating to the experiments described in Chapter 5 for Scots pine at different photon flux densities (figures 10.2a+b). It is interesting, considering the likely errors that could be incurred, that the decline in the absolute values of dA/dg_s at lower values of Q are consistent, and the trends with D are similar, even though the experiments for different levels of Q were done independently. Thus equation 10.6 appears to be fairly resiliant to errors in the estimation of m and E_m. The resultant values of dE/dA increase markedly at lower levels of Q. This implies that dE/dA is not constant when changes in A are caused by changes in Q. This is a result of the different shapes for the light response curves of g_s and A.

The dE/dA curves for the experiments reported in Chapter 7 (figures 10.3a+b), for different temperatures, reflect the responses of g_s to D (fig. 7.1). As for the Sitka spruce shoots in figures 10.1a+b, dA/dg_s increased with D, but there was still a decline in dE/dA. The differences in the dA/dg_s curves for the different temperatures is a complex function of a slightly different stomatal response to D, the temperature response of g_s and the temperature response of A. Although the response of E/A as a function of D appeared to overlap for the different temperatures, as was shown in Chapter 7, this does not result in the curves for dE/dA overlapping.

The dE/dA curves for Sitka spruce described in Chapter 9 (fig. 10.4b), show a completely different response, at comparable water potentials, to those shown in fig. 10.1b or that at 20 ^{o}C in fig. 10.3b for the same species. This is probably the result of the much weaker response of g_s to D found for these plants, although it may be, in part, a consequence of the different models used to describe the data (see below). The values of dE/dA can still not really be described as constant. This trend for dE/dA to increase at lower water potentials, is the opposite of that reported by Farquhar *et al* (1980b) for *C. avellana*, but this difference may be explained by the large, direct effect of water potential on A, that they also reported.

The type of model used to describe the physiological responses of g_s and A to D is likely to be quite important to the resultant shape of the dE/dA curve, particularly if one is going to extrapolate outside the range of measurement. Choosing a model with a strong physiological basis, such as that used for the data in Chapter 9, is likely to give the most realistic curves. In the case of the experiments described in Chapters 3, 5 and 7 there is not enough information to fit such a model, so a descriptive curve was used. Further investigation, with a more extensive data set, to test how different models perform is required. However, several of the studies that showed constant dE/dA, also used descriptive models (Farquhar *et al*, 1980b; Meizner, 1982).

Thus only two of the graphs, those for lodgepole pine and hybrid larch, can be considered as supporting evidence for the hypothesis that dE/dA remains constant. Of course, it is possible to discount the other responses on grounds of large errors in estimating dE/dA, or over-simplification of the calculation of dE/dA. It can also be argued that measurement of dE/dA in an assimilation chamber has affected the response and in addition, that the plants were subjected to a range of environmental conditions that they do not normally experience, in the field. However, similar arguments can be applied to many of the data presented in the literature that purports to show that dE/dA remains constant.

The predicted responses of g_s and E shown in figures 10.5 and 10.6 for fixed values of dE/dA indicate that only for the lowest value of λ is there a requirement for a stomatal response to D that causes a decline in E as D is increased beyond a certain limit. The general shapes of all of the predicted responses for $\lambda > 100$ are not greatly different from those reported here for the various experiments, i.e. the response of g_s is curvilinear with respect to D and results in E either increasing or reaching a plateau. The predicted value of A, shown in fig. 10.7, declines more or less linearly with D in a similar manner to the observed results. Thus the predicted curves are not very different from those found, even though the calculated values of dE/dA for the data are generally not very constant.

It is interesting to look at the extrapolation of the predicted curves to D=0. To maintain constant dE/dA, g_s tends towards infinity as D becomes smaller; although the curves for E and A follow a more realistic course. This shows that it is unlikely that a plant could maintain constant dE/dA when D is very small and leads to the question of, under which conditions it is important for the plant to control dE/dA to a constant value.

As shown by Williams (1983), plants in the field may, often, not follow an optimal response closely, either because it is physically impossible for them to do so, e.g. because they cannot have infinite g_s, or because there are other more important factors involved in their survival, for example the requirement to fix CO_2, irrespective of the amount of water lost, to allow flowering before the end of a season. For well-irrigated plants, e.g. marshland plants, there may be very little selective pressure for dE/dA to remain constant.

In conclusion, if one finds that dE/dA is constant, then one can say that there is evidence that the stomata do follow an optimal response. However, there may be many reasons why a plant will not perform optimally and in addition there are many problems in estimating dE/dA accurately. Perhaps a more realistic approach to testing the hypothesis is as done by Williams (1983), i.e. to compare the actual integral amount of water lost, per unit amount of CO_2 fixed over a period of time, with that expected if the stomata maintain dE/dA constant. However, this then requires a detailed model that includes realistic environmental variation and also some estimate of the dynamic responses of g_s and A. The simplicity of the whole concept of "optimal responses" is then lost. However, the hypothesis would then give us a scale upon which to judge the integrated performance of a plant.

CHAPTER 11

GENERAL DISCUSSION AND CONCLUSIONS

11.1 Direct responses of the stomata to D?

In none of the experiments reported here was there any evidence for a decline in E at high levels of D. Thus there is no requirement to invoke a mechanism for a direct response of the stomata to D, as discussed in Chapter 1. Whether one might find evidence for a direct response to D for other plants of the same species in the field, or for other conifers in general is hard to tell.

Problems in the analysis of field data, as discussed in Chapter 3, cast doubt on some of the evidence for a direct response of g_s to D, reported in the literature for conifers. Several of the experiments may, in retrospect, also suffer from technical problems, as was discussed with respect to porometer measurements in Chapter 3 and as described for a laboratory system in Chapter 6. It is also possible that the way in which experiments have been done may have influenced the measured response to D (see Chapter 4). Taking these factors into consideration the evidence for a direct response of g_s to D, in conifers is not very strong. There is considerable evidence to suggest a lesser degree of response in most coniferous species, but as found in the various experiments on Sitka spruce in this thesis, the degree of response can vary with shoot age and different plant material.

It is likely that similar problems in experimental technique and design may also have affected many of the responses of g_s to D that have been measured in non-coniferous species. In particular, in some experiments, the responses of g_s have been measured outside the normal growth conditions for the species and although a strong response to D may result, it may have little relevance to plant growth in the field. However, the evidence for a direct response is much stronger for some of these species. In particular the series of experiments done by Lange *et al* (1971) and Lösch (1979a+b) on the isolated epidermis of *Polypodium vulgare* L. showed that the stomata of this species are capable of responding

directly to D, at least *in vitro*, though possibly not to the same degree in the intact leaf (Edwards & Meidner, 1978).

The experiments in this thesis do not disprove the existence of a direct response to D, but show that not all species respond directly to D. In fact, when studying the literature it seems likely that species that respond directly to D are in the minority, not the majority as may have been thought in the mid-seventies. Some of the surveys of known responses of g_s to D found in the literature, e.g. Sheriff (1977), Lösch (1979a), show roughly equal numbers of species with either no response to D, some response to D and those with a direct response to D. However, these surveys are rather biased, as direct responses to D tend to be published more often because they are of more interest to the stomatal physiologist.

The type of response of g_s to D reported in this thesis is nonetheless important in the water balance of the plant in the field. If a plant can limit water loss to a maximum, as was shown for Sitka spruce in Chapter 3, then severe drops in leaf water potential will be uncommon. In addition, if water potential does drop significantly, a build up of stress hormones such as abscisic acid (Blake & Ferrell, 1977) will reinforce feedback to prevent further water loss.

It seems probable that a direct response of g_s to D, may be most important in terms of plant water use, for plants growing in arid environments where water is not freely available (Schulze *et al*, 1972; Schulze & Hall, 1982). Such a response allows the plant to prevent excessive loss of water, well before any drop in water potential might occur. However, there is a cost to the plant in responding in this way in terms of increased stomatal limitation of A. Therefore, it is not suprising that coniferous species, from temperate regions where water is rarely short, do not exhibit a direct response. However, Sheriff (1977) was unable to correlate the natural growth environment of plants he surveyed, with a direct response of g_s to D. It is probable though, that some of the direct responses to D that he measured for temperate plants, may well have subjected those plants to conditions they are unlikely to experience in the field.

11.2 A mechanism for the stomatal response to D

The original idea that the stomatal response to D was mediated by changes in bulk leaf water potential is not adequate to explain the response of g_s to D reported in this thesis, as the concurrent changes in water potential were small. It is not, however, necessary to invoke a direct response to D, but simply a response with a high degree of negative feedback (Jones, 1983). Nonetheless such a mechanism may be important in other species, particularly those which exhibit a decline in E as D increases. Therefore, rather than proposing a mechanism for stomatal response to D which simply covers the responses of the plants reported here, a general model will be presented.

The concensus of opinion (Lösch & Tenhunen, 1981) is that the response of g_s to an increase in D is mediated by direct water loss from the guard cells, or associated cells, which causes, at least in the short term, a change their turgor (generally termed a hydropassive change). Some minutes later, changes in potassium, malate, pH and starch contents are observed in the guard cells; factors which are normally associated with the active process of stomatal movement (hydroactive processes) in response to other variables such as a reduction in photon flux density or an increase in C_i (Willmer, 1983). The mechanism by which the initial changes in turgor stimulate the changes in biochemical processes is uncertain. The way in which a change in D causes a change in the turgor of the guard cells is a much discussed subject, e.g. Sheriff (1979), Jones (1983), Maier-Maercker (1983).

Several hypotheses have been proposed. They all have two common characteristics:

i) that there is substantial evaporation from the cells around and including the guard cells, termed 'peristomatal' water loss.

ii) that there is a substantial resistance to water flow in the pathway from the xylem vessels to guard cells, which in combination with 'peristomatal' water loss will cause the water potential of the guard cells to be lower than the bulk water potential of the leaf

and very sensitive to changes in the rate of peristomatal transpiration.

There is a reasonable amount of evidence to support the existence of both of these charactersitics. Evidence for the high rate of peristomatal transpiration can be found in the studies of Meidner (1976b) and Tyree & Yianoulis (1980). Meidner purported to show up to 40% of the water lost from a leaf might be lost from the internal surface of the epidermis. Tyree & Yianoulis (1980) have disputed Meidner's exact figures and state that over 75% of the water lost may originate from around the stomata, if not from the guard cells themselves. However, both sets of workers used very simple leaf structure models and one must be cautious in extrapolating their exact values to other species.

Evidence for the second characteristic is also found in Tyree & Yianoulis (1980). They propose higher resistances to water flow in the epidermis than those proposed by Sheriff & Meidner (1974). However, both studies claim that the resistance is high enough to cause substantial drops in water potential between the xylem vessels and the guard cells. Tyree & Yianoulis (1980) calculated, using their estimates of epidermal conductance and assuming that the majority of transpiration occurs from the internal surfaces of the guard cells, that the difference could be of the order of -2.0 MPa, at only moderate levels of D. Sheriff (1982) presented further measurements of epidermal conductivity and, although they tend to confirm the lower values of conductivity of Sheriff & Meidner (1974) for a species whose stomata do not respond to D, he found significantly lower values for a species that does.

If large changes in guard cell water potential can occur, without changes in bulk leaf water potential, as a result of peristomatal transpiration and a high resistance to water movement, then this would provide the high degree of negative feedback required to explain the responses of g_s to D, measured in this thesis (see above).

To explain a direct response of g_s to D, it is necessary to introduce the possibility that there is a site of water loss external to the leaf. This possibilty was initially proposed by Seybold (1961/62). However, the

idea was not given full consideration until the experiments of Lange *et al* (1971) showed that stomata could respond directly to D in isolated epidermal strips. More recently, Cowan (1977) and Farquhar (1978) showed theoretically, that if the stomatal response to D is mediated by steady-state fluxes of water loss and if the total water loss from a leaf (E) declines as D increases as a result of stomatal closure, there must be a source of water loss which is independent of g_s, i.e. transpiration from the outer surface of the leaf.

There is considerable evidence to show that external peristomatal water loss may be significant. All plant cuticles are, to a degree, permeable to water vapour (Martin & Juniper, 1980; Schönherr, 1982), but in a series of papers by Maier-Maercker, summarised in Maier-Maercker (1983), it has been shown, using a range of techniques, that the water loss from around the stomatal complex is high. Particular areas of likely water loss have also been proposed for other species, based on histochemical tests of the cell walls (Ng, 1978; Edwards & Meidner, 1978; Appelby & Davies 1983a+b). However, these tests alone may be misleading as leaf surfaces are often covered in a layer of wax which usually has hydrophobic properties. In particular the leaf surface of *P. sylvestris* is generally covered with an amorphous layer of wax, with an overlying crystalline structure.

The simplest model involving peristomatal transpiration is that the water loss causes a drop of turgor in the guard cells and thus stomatal closure. The external source of water loss could even be the guard cells themselves. However, in the simplest form, this hypothesis is rather naive because one cannot consider the turgor relations of the guard cells alone, as they interact with the cells around them. It was shown by Edwards *et al* (1976) that the subsidiary cells of *Tradescantia virginiana* had a mechanical advantage over the guard cells and, as a result, changes in stomatal aperture were not directly correlated to the absolute turgor pressure in the guard cells, but to the difference in turgor between the guard cells and the subsidiary cells. Similar findings have been made for other species and these effects have been considered with respect to models of the mechanics of stomatal opening (Sharpe & Wu, 1978; Wu & Sharpe, 1979). The species studied and models developed to date, have been limited to species whose stomata have relatively simple structure, so

one must be careful not to extrapolate these findings too far when considering other species with different stomatal complexes.

Maier-Maercker showed, for some species, that external transpiration was higher from the subsidiary cells than the guard cells and that the external loss of water from the guard cells might even decline as they close. Following the arguments of Edwards *et al* (1976), as D increases for these species, one would expect a greater turgor loss in the subsidiary cells than the guard cells and the stomata to open. Sheriff (1979) claimed that this often happens in reality, but is usually undetectable using gas-exchange techniques, as the active processes rapidly 'catch up' and close the stomata. A mechanism similar, to Sheriff's, has also been proposed by Maier-Maercker (1983). She calls this a mechanism of 'influx-efflux' control. If D is increased then water loss from the stomatal complex will change. Various lags in the pathway from the xylem vessels will cause temporary changes in the turgor of the guard cells which will trigger off the active processes to change g_s.

However, both mechanisms are non-steady-state, i.e. they only explain the short-term response to rapid changes in D. The only way they might act in a pseudo-steady-state manner is by the involvement of the production of a stress hormone in the epidermis, which might stabilise the change in g_s (Sheriff, 1979). These proposed mechanisms may not be limited to species with significant rates of external peristomatal transpiration, as if the change in D is large, temporary changes in epidermal turgor could occur through changes in the rates of water loss from sites within the leaf. Thus, whilst these proposed mechanisms may be important in explaining responses to large steps in D, such as those reported in Chapter 4, they cannot explain steady-state direct responses of g_s to D, where D has been increased slowly.

In my opinion, both the concepts of mechanical advantage of the subsidiary cells and the relative rates of peristomatal transpiration from different cells of the epidermis, have been applied too widely. For some species, including conifers, the subsidiary cells are not, physically, in a position to exert the same degree of mechanical advantage over the guard cells in the way conceived for guard cell complexes already studied (Sharpe

& Wu, 1978). It is also likely that the distribution of cuticular water loss will also vary markedly between species and, possibly, even with leaf age and growth conditions. Thus the exact mechanism of the response to D will vary from species to species.

One possibilty, that is not often considered, is that the external sites of water loss may be close to sensitive membranes important in the hydroactive processes of stomatal movement. Thus small, local changes in turgor, at these sites, might cause stomatal response without involving the mechanical interactions discussed above. However, although this mechanism may be important in instances, it cannot explain the findings of Lösch (1977) where closure precedes changes in the hydroactive processes in response to an increase in D.

Based on the arguments above and on anatomical evidence in the literature, it is possible to piece together an electro-chemical analogue that is adequate to represent the main features of the system (fig. 11.1). This diagram is an extension of that originally described by Raschke (1970) and later by Ng (1978) and Jarvis (1980). The symbols used in the diagram represent the following fluxes and resistances:

r_{wm1} and r_{wm2} represent two components of water flow through mesophyll tissue. Two components are likely as, after passing through the tissue surrounding the xylem vessels, including the bundle sheath and some mesophyll cells with resistance r_{wm1}, the pathways of water movement to a) the epidermal sites of water loss and b) the mesophyll sites of water loss, are likely to be different. r_{wm1} is shown as possibly being variable as, in some coniferous species, there is evidence for the presence of an endodermis around the the xylem (Esau, 1960). If this endodermis acts in a similar manner to that found in the roots, then its resistance to water flow may vary substantially.

r_{bse} represents possible direct pathways of water flow to the epidermis, found in some species, called bundle sheath or vein extensions. Although it has been proposed that these structures prevent the stomata responding to D in some species (Sheriff &

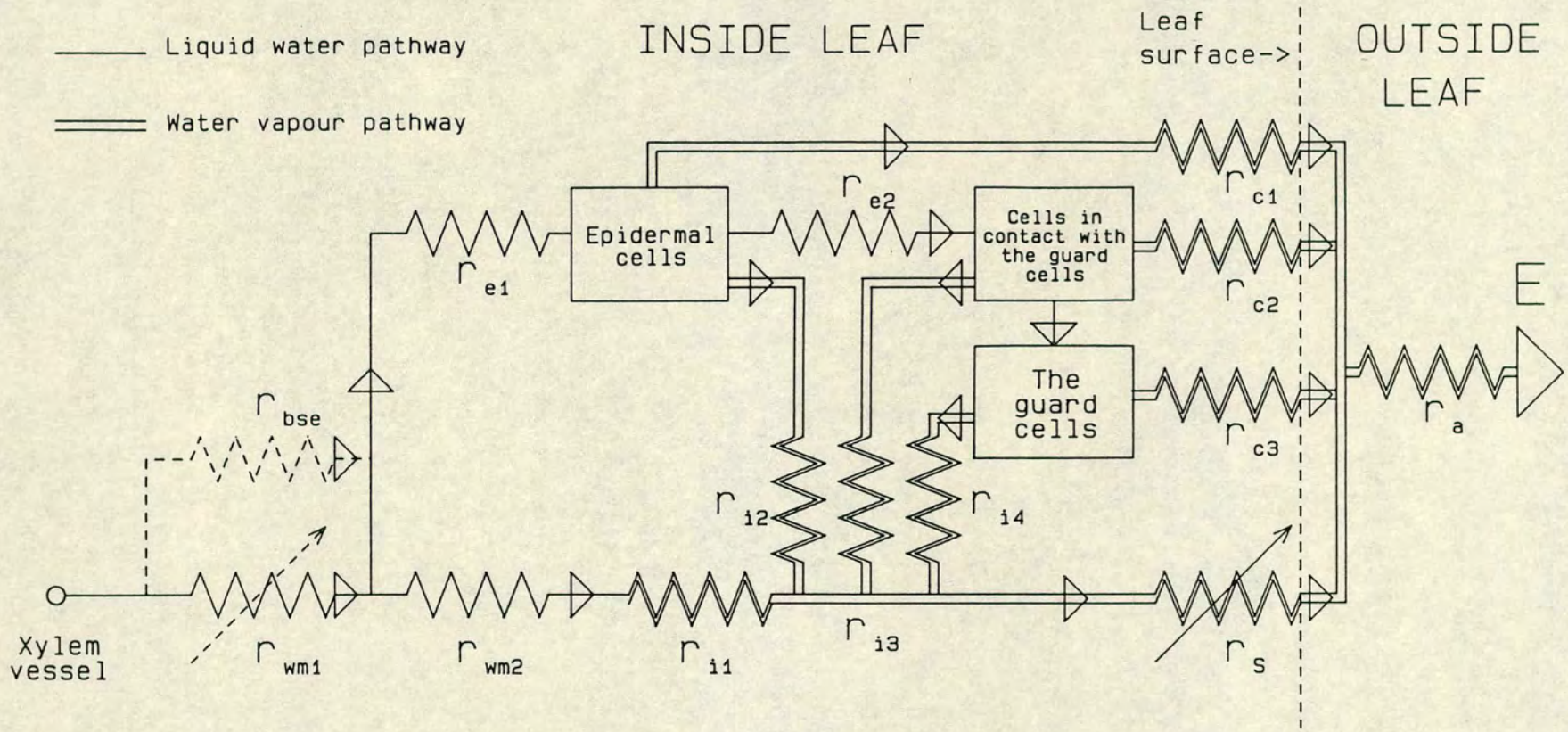

Figure 11.1: A theoretical diagram of water flow in a hypothetical leaf. See the text for a description of the symbols and fluxes.

Meidner, 1975), this was not confirmed in the survey performed by Sheriff (1977).

r_{e1} and r_{e2} represent resistances to water flow in the epidermis. Two components are included, firstly to allow for the existence of the hypodermis in some conifers (Esau, 1960). This would be in the position of r_{e1} and may be a substantial resistance to water flow. Secondly to emphasise that the unspecialised epidermal cells may also lose substantial quantities of water externally, particularly as in many species the water may have to pass through many epidermal cells before reaching the stomatal complex.

r_{i1}, r_{i2}, r_{i3} and r_{i4} represent resistances to gaseous diffusion of water vapour from internal sites of evaporation to the stomatal pore. Although these resistances are generally considered to be insignificant, this may not be the case in some species; and in particular r_{i1} may be substantial (Tyree & Yianoulis, 1980; Jones, 1983).

r_{c1}, r_{c2} and r_{c3} represent the resistance to water vapour diffusion through the cuticle from various possible sites of evaporation to the exterior. The magnitude of these resistances is likely to vary with cell wall structure and wax deposition (Martin & Juniper, 1980).

r_s, r_a and E are as defined in Appendix 1.

In the diagram, water is shown to flow from the unspecialised epidermal cells to 'Cells in contact with the guard cells'. This term is used as in some species there is a complex of cells around the guard cells, e.g. in *Commelina* sp., some of which are not traditionally called subsidiary cells. These cells are considered separately from other epidermal cells as they may have a mechanical interaction with the guard cells. No resistance to flow is shown between these cells and the guard cells as it is likely that this resistance will be small. However, some workers dispute this (Maier-Maercker, 1983) so the possibilty cannot be ruled out.

Whilst this diagram is intended primarily to show the complexity of water flows through the system, it is possible to relate various components of it to some of the proposed mechanisms, discussed above. If, for a plant there is no evidence for a decline in E as D increases then, the fluxes through the cuticle may be insignificant, the response being caused by water loss, via the stomata, from internal peristomatal water loss. If there is a steady-state, direct response to D, then at least one of the cuticular components must be significant. However, it should be noted that if most of the water lost from a leaf evaporates from the internal surfaces of the guard cells, then external loss from the guard cells alone is unlikely to cause the direct response, as stomatal closure would reduce the much larger loss from the internal surfaces and thus lead to rehydration of the guard cells.

One further complication has been added to this system recently by Appleby & Davies (1983a+b) who suggest that during stomatal closure the guard cells of some species may move in such a way so that they present surfaces, normally internal to the leaf, with high permeability to water loss, to the outside of the leaf. This could explain the direct response of g_s to D and also the reluctance of the stomata to open after exposure to high values of D. However, it remains to be seen whether such movements are common or if they happen during the short term response to D. It seems more likely they may play a role in the response to long term water stress.

To offer an explanation of changes in the response of g_s to D at different levels of water stress requires yet further assumptions to be made. One possible approach considered by Ng (1978) and similarly by Jarvis (1980) has been to apply the concept of a Höffler diagram to the guard cells. To apply the diagram to explain stomatal responses, they had to make several assumptions. One of these was the relationship between the turgor difference between the guard cells and subsidiary cells and stomatal opening. As discussed above, taking such relationships from studies in the literature and applying them to species with markedly different stomatal complexes, such as conifers, may be misleading. However, such an approach does offer a physiological base for

understanding the responses.

One aspect of the model shown in fig. 11.1 is that if the xylem water potential goes down, then one would expect the guard cell water potential to drop by a similar amount, if all of the hydraulic resistances are constant. Thus, taking a simplistic view, one would expect the effects of changes in xylem water potential and changes in guard cell potential, caused by changes in D, to be additive. However, in reality several factors alter this response. Firstly, at very high water potentials and negligible transpiration rates, it is possible that the potential of the guard cells will be above a threshold for closure (Jarvis, 1980). As the guard cell water potential drops further it is unlikely that the stomata will close linearly with respect to water potential because of the mechanics of stomatal opening (Sharpe & Wu, 1978). Secondly, it is likely, that even in species which exhibit a direct response to D, that evaporation from the internal surfaces of the epidermis plays an important role in determining the water potential of the guard cells. Thus as the stomata close in response to bulk water potential this source of water loss will be reduced, although not necessarily directly in proportion to g_s (Sheriff, 1982). Consequently the stomata will become less sensitive to D as they close. This hypothesis fits in neatly with the findings in this thesis and those Morison & Gifford (1983), that dg_s/dD is generally proportional to g_s.

Although it is possible to propose mechanisms by which the stomata might sense D, the exact details of how the mechanisms may work together in a leaf requires a greater understanding of the water relations of the epidermis and the mechanics of stomatal opening than we have at present. It is also unlikely that one overall mechanism can be applied to all plants, because of anatomical variation found amongst leaves of different species. Even if we can understand how the stomata initially sense D, we are then faced with the question of how small changes in guard cell turgor cause consequent changes in the biochemical processes associated with stomatal opening. There is some evidence that changes in turgor can affect membrane permeability (Maier-Maercker, 1983), but how these changes in permeability tie in with the mechanisms of response of the stomata to other variables, is not understood. Clearly much more

research is required before we fully understand the stomatal response to humidity.

APPENDIX 1

A LIST OF THE SYMBOLS, UNITS AND ABBREVIATIONS

Symbols and their definitions

Symbol	Definition
a	Parameter in equation 3.1/8.2 for the initial slope of the E/D relationship.
A	Net assimilation rate <=> net CO_2 flux.
A_g	Gross assimilation rate (as defined by Watts & Neilson, 1978).
A_i	The value of A for D=0, i.e. the intercept value on the y-axis for a linear regression of A *versus* D.
A_m	Parameter in equation 9.1 for the asymptotic value of A with respect to C_i.
A_o	The value of A when $C_i = C_a$, $g_{tc} = \infty$.
b	Parameter in equation 8.2 for the water potential when $g_s = 0.5\ g_{max}$.
c	Parameter in equation 8.2 controlling the shape of the response of g_s to water potential.
C_a	Ambient CO_2 mole fraction.
C_b	Mean of C_a and C_i.
C_e	CO_2 mole fraction entering the chamber.
C_i	Internal, intercellular space CO_2 mole fraction.
C_o	CO_2 mole fraction coming out of the chamber.
D	Leaf-to-air vapour pressure difference.
E	Transpiration rate.
E_m	Parameter in equation 3.1 for the maximum E.
e_a	Ambient water vapour partial pressure.
e_e	Water vapour partial pressure entering the chamber.
e_i	Internal water vapour partial pressure of the leaf, <=> to the saturated vapour pressure at leaf T.
e_o	Water vapour partial pressure coming out of the chamber.
F_e	Molar flow of air entering the chamber.
F_o	Molar flow of air coming out of the chamber.
g_a	Boundary layer conductance to water vapour.
g_{ac}	Boundary layer conductance to CO_2.

g_m	Mesophyll conductance.
g_{max}	Parameter in equation 8.1 for the value of g_s when $\psi_{xyl} = 0$.
g_s	Stomatal conductance to water vapour.
g_{sc}	Stomatal conductance to CO_2.
g_t	Total conductance to water vapour.
g_{tc}	Total conductance to CO_2.
L	Plan leaf area.
m	The slope of a linear regression for A *versus* D.
P	Atmospheric pressure.
Q	Incident photon flux density.
r_s	Stomatal resistance to water vapour.
r_{sc}	Stomatal resistance to CO_2.
r_m	Mesophyll resistance.
r_w	Total plant resistance to water vapour (Whiteman & Koller, 1964)
R_l	'Light respiration' rate, in equation 9.3.
t	Time
T	Temperature.
w_a	Ambient water vapour mole fraction.
w_b	Mean of w_e and w_i.
w_e	Water vapour mole fraction entering the chamber.
w_i	Internal water vapour mole fraction of the leaf, <=> to the saturated mole fraction at leaf T.
w_o	Water vapour mole fraction coming out of the chamber.
α	The initial slope of the A/C_i relationship in equation 9.1.
Δw	Leaf-to-air water vapour mole fraction difference.
Γ	CO_2 compensation point as a mole fraction.
λ	A constant representing constant dE/dA.
ψ_{xyl}	Xylem water potential.
θ	The convexity parameter in equation 9.1.
l_g	relative stomatal limitation of A

Symbols and their units

The units, as commonly used in this thesis, are given below. The magnitude may vary with use. Symbols not listed represent dimensionless quantities. The 'wildcard' subscript 'x' represents one of several possible subscripts (see above).

Symbol	Base unit
a	mmol m^{-2} s^{-1}
A_x	µmol m^{-2} s^{-1}
b	MPa
C_x	µmol mol^{-1} (<=> dimensionless X 10^6)
D	kPa
E_x	mmol m^{-2} s^{-1}
e_o	kPa
F_x	mol s^{-1}
g_x	mol m^{-2} s^{-1}
L	m^2
m	µmol m^{-2} s^{-1} kPa^{-1}
P	kPa
Q	µmol m^{-2} s^{-1}
r_x	m^2 s mol^{-1}
R_l	µmol m^{-2} s^{-1}
t	s
T	^oC
α	mol m^{-2} s^{-1}
ψ_{xyl}	MPa

Abbreviations

Abbreviation	Meaning
a.s.d.	asymptotic standard deviation
R.H.	relative humidity
s.e.	standard error

APPENDIX 2

A list of the 'common' English names used for coniferous species in this thesis and their scientific, latin equivalents

Common name	Scientific name
Aleppo pine	*Pinus halepensis* Mill.
Douglas-fir	*Pseudotsuga menziesii* (Mirb). Franco
Engelmann spruce	*Picea engelmannii* Engelm.
European larch	*Larix decidua* Mill.
grand fir	*Abies grandis* (Dougl.) Lindl.
hybrid larch	*Larix* X *eurolepis* Henry.
incense-cedar	*Calocedrus decurrens* (Torrey) Florin
lodgepole pine	*Pinus contorta* (Dougl.) Loud.
noble fir	*Abies procera* Rehd.
Norway spruce	*Picea abies* (L.) Karst
ponderosa pine	*Pinus ponderosa* Laws.
radiata pine	*Pinus radiata* D. Don
Scots pine	*Pinus sylvestris* L.
Sitka spruce	*Picea sitchensis* (Bong.) Carr.
western hemlock	*Tsuga heterophylla* (Raf.) Sarg.
white fir	*Abies concolor* (Gord.) Hildebrand

APPENDIX 3

A LIST OF EQUIPMENT MANUFACTURERS

Addresses are in the U.K, unless stated otherwise.

Air pumps	Charles-Austin, Byfleet, Surrey.
Temperature controllers	Eurotherm, Worthing, Sussex.
Dew point hygrometers	EG & G, Cambridge Instruments, from Auriema, Slough, Bucks.
Data logging equipment	Solatron Electronics, Farnborough, Hants.
Gas cylinders	Rank Hilger, Margate, Kent.
Gas couplings	Bel-Art from MacKay & Lynn Ltd, Edinburgh.
Gas diluter	Analytical Dev. Co. Ltd., Hoddesdon, Herts.
Gas tubing	Samuel Moore & Co. Ltd., Coventry, CV6 6FJ.
Gas-mixing pumps	Wosthoff oHG, D463 Bochum, FRG.
Heating tapes	Hotfoil Ltd., Wolverhampton, Staffs.
Humidity sensors	Vaisala OY, Helsinki, Finland.
Infra-red gas analyser	Hartmann & Braun, Moulton Park, Northampton.
Leaf area meter	Li-Cor, Lincoln, Nebraska, U.S.A.
Light Sources	Wotan Lamps Ltd., London SW19 8HU.
Mass flowmeters	Brooks, Emerson Electric Co.Ltd, Stockport.
	Tylan, Epak Electronics, Reigate, Surrey.
Peltier Cooling Device	Cambion, Castleton, Yorkshire SBO 8WR.
Precision P.R.T.	Guildline, Canada.
Pressure bomb	Dept. of Forestry & Nat. Res, Univ. of Edinburgh.
Rotameter flowmeters	GEC-Elliot, Margate, Kent.
Solenoid valves	Schraeder, from Graham Boyd & Co., Edin.
Thermocouple reference	Mectron (Frigistor), Slough, Bucks.
Quantum sensor	Li-Cor, Lincoln, Nebraska, U.S.A.
Water bath	Grant Instruments Ltd, Cambridge.
Water vapour generator	Analytical Dev. Co. Ltd., Hoddesdon, Herts.

APPENDIX 4

Details of the computer packages used for the data analysis and presentation in this thesis.

i) **BMDP, PAR:** Derivative-free, least-squares, non-linear regression package.
Author: M. Ralston
Reference: BMDP Statistical Software (1981), Ed. W.J.Dixon, UCLA Press.

ii) **Dplayout:** Word processing package, for Phillips GP300 printer.
Author: J.M.Murison (originator H.Dewar, Computer Science)
Reference: User Notes 42 & 43, Edinburgh Regional Computing Centre, J.C.M.B, Kings Buildings, Mayfield Road, Edinburgh.

iii) **Easygraph:** Graph plotting package.
Author: N.Stroud (originator W.A.Watson)
Reference: User Note 12, Edinburgh Regional Computing Centre, J.C.M.B, Kings Buildings, Mayfield Road, Edinburgh.

iv) **Genstat:** Statistical package, version IV.03
Author: Various
Reference: Manual available from - The Statistics Department, Rothamsted A.F.R.C Experimental Station, Harpenden, Herts.

v) **Presto:** General modelling, graph plotting and stat's package.
Author: R.I.Muetzelfeldt
Reference: Available from the author at the Department of Forestry & Natural Resources, University of Edinburgh, EH9 3JU.

APPENDIX 5

A listing of the program used to simulate the errors caused by the Model 880 dewpoint meter.

```
C A program to model the limitations of the 880 dewpoint meter
C when used in the gas analysis system for gs vs. D studies

C List of variables
C APPGS = Apparent gs
C APPEE = Apparent water vapour pressure entering the chamber
C APPEO = Apparent water vapour pressure entering the chamber
C ATP = Atmospheric pressure
C EE = The actual, calculated water vapour pressure entering the chamber
C EI = Saturated water vapour pressure at leaf temperature
C EMIN = The lower limit of water vapour pressure measurable
C        by the 880 dewpoint meter.
C EO = The actual, calculated water vapour pressure entering the chamber
C D = The leaf-to-air water vapour pressure difference
C FL = The ratio of flow to leaf area
C REALE = The actual, calculated E
C REALGS = The actual calculated gs
C STARTFL = The initial input value of flow/leaf area
C STEPD = The incremented value of D

C Set up the arrays
      DIMENSION D(80),FL(80),EO(80),EE(80),APPGS(80),REALE(80),APPE(80)

C Define the constants
C For leaf temp = 20 oC
      REALGS=0.208
      EI=2.373
      STARTFL=0.4
      ATP=101
      EMIN=0.2837

C Assuming that gs and FL are constant then calculate EE
      NR=1
      DO 001 STEPD=0.01,EI,0.05
        D(NR)=STEPD
        FL(NR)=STARTFL
        EO(NR)=EI-D(NR)
        EE(NR)=EO(NR)-(REALGS*D(NR)/FL(NR))

C If EE is negative (impossible!) then set EE to zero and increase FL
        IF(EE(NR).LT.0)THEN
          EE(NR)=0
          FL(NR)=REALGS*D(NR)/EO(NR)
        ENDIF
        NR=NR+1
 001  CONTINUE
      NR=NR-1

C Assuming that the minimum water vapour pressure the dewpoint meter
```

```
C will read is EMIN, then calculate the apparent E and gs .
      DO 002 I=1,NR

C First calculate the real E
        REALE(I)=1000*(EO(I)-EE(I))*FL(I)/ATP

C Impose the minimum limits of the water vapour pressures EO and EMIN.
        IF(EO(I).LT.EMIN)THEN
          APPEO=EMIN
        ELSE
          APPEO=EO(I)
        ENDIF
        IF(EE(I).LT.EMIN)THEN
          APPEE=EMIN
        ELSE
          APPEE=EE(I)
        ENDIF

C Now calculate the apparent E and gs.
        APPE(I)=(APPEO-APPEE)*FL(I)/ATP
        APPGS(I)=APPE(I)*ATP/D(I)

C Convert E to mmol
        APPE(I)=APPE(I)*1000

 002  CONTINUE

C Write out the results to a file
      WRITE(10,101)(D(I),REALGS,APPGS(I),REALE(I),APPE(I),I=1,NR)
 101  FORMAT(5F10.4)

      STOP
      END
```

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